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CONTENTS

PAGE

I. Nesbitt, Beattie. On the Presence of Cholin and Neurin in the Intestinal Canal during its Complete Obstruction.—A Research on Autointoxication. . . 1

II. Richardson, Mark Wyman. On the Value of Urotropin as a Urinary Antiseptic with Especial Reference to its Use in Typhoid Fever. 19

III. Baer, W. S., Dawson, P. M., and Marshall, H. T. Regeneration of the Dorsal Root Fibres of the Second Cervical Nerve within the Spinal Cord. 29

IV. Bliss, C. L., and Novy, F. G. Action of Formaldehyde on Enzymes and on Certain Proteids. 47

V. Thayer, William Sydney, and Lazear, Jesse William. A Second Case of Gonorrhœal Septicæmia and Ulcerative Endocarditis with Observations upon the Cardiac Complications of Gonorrhœa 81

Plate I.

VI. Herter, C. A., and Wakeman, A. J. On Alterations in the Composition of the Blood Resulting from Experimental Double Nephrectomy. 117

VII. Meltzer, S. J., and Norris, Charles. On the Influence of Fasting upon the Bactericidal Action of the Blood. 131

VIII. Hibbard, Cleon Melville, and Morissey, Michael J. Glycosuria in Diphtheria. 137

IX. Howard, W. T., Jr. Hæmorrhagic Septicæmia in Man Due to Capsulated Bacilli. 149

PAGE

X. Curry, Joseph J. Bacillus Capsulatus (Bacillus Pneumoniæ of Friedlaender?) with Especial Reference to its Connection with Acute Lobar Pneumonia. . . . 169

XI. Cabot, Follen. Report of Experimental Work on the Dilution Method of Immunization from Rabies. . . 181

XII. Nichols, Joseph Longworth. A Study of the Spinal Cord by Nissl's Method in Typhoid Fever and in Experimental Infection with the Typhoid Bacillus. . . 189
Plates II-IV.

XIII. Smith, Theobald. The Thermal Death-point of Tubercle Bacilli in Milk and Some Other Fluids. . . 217

XIV. Stewart, G. N. The Changes Produced by the Growth of Bacteria in the Molecular Concentration and Electrical Conductivity of Culture Media. 235
Plates V-VIII.

XV. Knox, J. H. Mason, Jr. On Supra-arterial Epicardial-Fibroid Nodules. 245
Plates IX-XI.

XVI. Hektoen, Ludvig. The Organism in a Case of Blastomycetic Dermatitis. 261
Plates XII-XV.

XVII. Opie, Eugene L. A Case of Hæmochromatosis.—The Relation of Hæmochromatosis to Bronzed Diabetes. 279

XVIII. Herter, C. A., and Wakeman, A. J. The Action of Hepatic, Renal and Other Cells on Phenol and Indol under Normal and Pathological Conditions. 307

XIX. Cushny, Arthur R. On the Interpretation of Pulse-tracings. 327
Plates XVI and XVII.

XX. Adami, J. George; Abbott, Maude E., and Nicholson, F. J. On the Diplococcoid Form of the Colon Bacillus. 349
Plates XVIII-XX.

PAGE

XXI. SMITH, THEOBALD. The Relation of Dextrose to the Production of Toxin in Bouillon Cultures of the Diphtheria Bacillus. 373

XXII. TAYLOR, ALONZO ENGLEBERT. The Origin of Fat from Protein in the so-called Fatty Metamorphosis of Phosphorus Poisoning. 399

XXIII. MACCALLUM, JOHN BRUCE. A Contribution to the Knowledge of the Pathology of Fragmentation and Segmentation, and Fibrosis of the Myocardium. . . 409

Plates XXI and XXII.

XXIV. WHITE, FRANKLIN WARREN. Cultures from the Blood in Septicæmia, Pneumonia, Meningitis and Chronic Diseases. 425

XXV. HEAD, GEORGE DOUGLAS, and WILSON, LOUIS BLANCHARD. A Case of Suspected Rabies with Isolation of Bacillus Diphtheriæ from the Central Nervous System. 451

XXVI. ELSBERG, CHARLES A. An Experimental Investigation of the Treatment of Wounds of the Heart by means of Suture of the Heart Muscle. 479

XXVII. MACCALLUM, WILLIAM G., and HASTINGS, THOMAS W. A Case of Acute Endocarditis Caused by Micrococcus Zymogenes (Nov. Spec.), with a Description of the Microorganism. 521

XXVIII. FISCHER, MARTIN H. A Study of the Neurone Theory. 535

Plates XXIII and XXIV.

XXIX. BRILL, N. E., and LIBMAN, E. A Contribution to the Subjects of Chronic Interstitial Nephritis and Arteritis in the Young, and Family Nephritis; with a Note on Calcification in the Liver. 541

Plate XXV.

XXX. LE COUNT, E. R. Lymphoma, a Benign Tumor Representing a Lymph Gland in Structure. 559

Plates XXVI and XXVII.

PAGE

XXXI. Oertel, Horst. Observations Concerning Leukæmic Lesions of the Skin. 569

XXXII. Philips, Carlin. A Case of Addison's Disease with Simple Atrophy of the Adrenals. 581
Plate XXVIII.

XXXIII. Hamilton, Alice. A Peculiar Form of Fibrosarcoma of the Brain. 597
Plates XXIX and XXX.

XXXIV. Fuller, George W., and Johnson, George A. On the Differentiation and Classification of Water Bacteria. 609

XXXV. Jordan, Edwin O. Bacillus Pyocyaneus and its Pigments. 627

XXXVI. Atkinson, James P. A Preliminary Note on the Fractional Precipitation of the Globulin and Albumin of Normal Horse's Serum and Diphtheric Antitoxic Serum and the Antitoxic Strength of the Precipitates. 649

Index. 651

VOLUME IV JANUARY, 1899 No. 1

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EXPERIMENTAL MEDICINE

ON THE PRESENCE OF CHOLIN AND NEURIN IN THE INTESTINAL CANAL DURING ITS COMPLETE OBSTRUCTION.

A RESEARCH ON AUTOINTOXICATION.

BY BEATTIE NESBITT, M. D.

(*From the Pharmacological Laboratory of the Johns Hopkins University.*)

In the normal processes of digestion the proteids and carbohydrates of our food are changed into more readily assimilated compounds, which are further altered before reaching the tissues; for example, the peptones,* which, if absorbed unaltered into the system would be very toxic, are changed into nutritive material in passing the intestinal wall. As a result of bacterial activity we may have these compounds broken up in a different manner, giving rise, either as immediate or terminal products of the decomposition of the proteid or carbohydrate molecule, to substances of more or less toxic character. Some of these substances, as phenol, the cresols, the dihydroxy-benzenes, indol and skatol, are known to occur as a result of the constant action of putrefactive bacteria in the large intestine. We may also have a large number of organic acids of the fatty series, as acetic, lactic, butyric, caproic, caprylic, etc., which have been shown to occur in various catarrhal conditions of the intes-

* According to E. Fiquet the poisonous effects usually ascribed to peptones and albumoses are in reality due to ptomaines or other toxins which have not been removed by the ordinary processes of purification. *Compt. rendus Acad. d. sc.*, 1897, p. 1371.

tinal tract, and whose irritating effects have been studied by Bókai.* Gases, as hydrogen sulphide, methylmercaptan,† carbon dioxide, methane and ptomaines, as putrescin (tetramethylendiamin),‡ cadaverin (pentamethylendiamin),‡ ethylidendiamin, are also more or less constantly present.

Opinions vary greatly on the toxicity of these substances, as the pharmacology of but one of them (phenol) has been carefully worked out.

For consideration in this respect they may be divided into three classes: (1) the fatty acids and the various gases, whose action in this connection is almost wholly irritative and need not be considered from the point of view of absorption, except perhaps in the case of infants; (2) substances of the aromatic series, which include phenol, the cresols, the dihydroxy-benzenes, indol and skatol, and (3) the diamins, including putrescin, cadaverin and ethylidendiamin. Substances of the second group are all excreted in the urine as conjugate or ethereal sulphates, and it is by their estimation that we judge of the extent of the putrefactive processes, more especially in the large intestine. From the fact that their molecules are of fairly simple structure, and in the case of most of them completely oxidized, we may consider the amount excreted as a reasonable index of the quantity absorbed.

With phenol (carbolic acid) we are well acquainted; its use as an antiseptic has for years been general, as a poison it has been taken in large quantities, sometimes without fatal results. It has been administered in various diseases, more especially those in which intestinal antisepsis was sought, as typhoid fever, in amount so much larger than the quantity produced in the intestine and absorbed that a comparison would be ridiculous. Thus Brieger § has found the amount excreted in 24 hours by a healthy individual to be about 15 milligrammes, while the textbooks on therapeutics set the maximum daily dose at 600 milligrammes. The cresols or methyl-hydroxy-benzenes are a later addition to the materia medica introduced by Laplace, and have undoubtedly many advantages over carbolic acid. It has been claimed that they are three times less toxic than phenol.|| I think from my own experiments that

* Bókai, *Arch. f. exp. Pathol. u. Pharmakol*, xxiii, 209; xxiv, 153.

† Nencki, *Jahresb. ü. Thier-Chemie*, xx, 309.

‡ Udránszky and Baumann, *Zeitschr. f. physiol. Chem.*, xiii, 562; xv, 77.
Werigo, Pflüger's *Archiv*, li, 362.
Roos, *Zeitschr. f. physiol. Chem.*, xvi, 192.
Garcia, ibid., xvii, 543.

§ *Zeitschr. f. physiol. Chem.*, ii, 241.

|| Charteris, *Lancet*, 1894, i, 801.

this figure is too high, but I have found that, while their action on blood-pressure and respiration presents the general phenol picture, recovery is more prompt. The injection of 50 cc. of 0.5 per cent sol. of cresol in normal saline solution into the jugular vein of a dog weighing 4 kilos caused the blood-pressure to fall 41 mm.; it returned to the normal in 13 minutes. Paracresol, according to Baumann and Brieger, occurs in largest amount among the members of this series, all of which give on distillation a reaction with bromine, the ortho- and meta-cresols occurring in traces.

The dihydroxy-benzenes—resorcin, hydroquinon and pyrocatechin—are present in the urine only in traces. All three have been used as medicines, their daily doses being respectively, 0.1-0.6 grammes, 0.25-1.5 grammes, and 0.3-0.2 grammes.

Possibly the substance which is attracting more attention than any of these at the present time is indol, which appears in the urine as indican, or indoxyl potassium sulphate. The amount of indican excreted is for an average man about 12 milligrammes per day; this is equivalent, if all is excreted that is absorbed, to an absorption of 6 milligrammes of indol in 24 hours. Experiments on the toxicity of indol and on its fate in the organism have been made by a number of investigators. Jaffé,* Nencki † and also Baumann ‡ administered considerable quantities of indol to dogs both by subcutaneous injection and by feeding, and although the object of these experiments was to determine the amount of indol that was converted into indican, it was at the same time observed that indol is not a toxic substance. Jaffé found no toxic symptoms following the subcutaneous injection of considerable quantities of indol prepared according to Baeyer's synthesis, and Nencki noted that a dog showed no signs of intoxication after receiving 1 gramme of indol by mouth, but with a dose of 2 grammes during 24 hours developed diarrhœa. Experiments more directly relating to the toxic influence of this substance were next undertaken by Christiani,§ who found that a fowl gave no signs of poisoning when it received 0.07 gramme of indol mixed with bread crumb, but that frogs reacted with decided symptoms in about an hour after the subcutaneous administration of from 1.2-2.4 milligrammes in solutions of 1:1000. The average fatal dose for frogs was 12 mmg. in 1 per cent solution subcutaneously administered. The symptoms were in general like those following the administration of phenol and need

**Centralbl. f. d. med. Wiss.*, 1872, No. 1.
† *Ber. d. deutsch. chem. Gesellsch.*, ix, 299.
‡ Pflüger's *Archiv*, xiii, 285, and *Ber. d. deutsch. chem. Gesellsch.*, ix, 54.
§ *Zeitschr. f. physiol. Chem.*, ii, 273.

not be given in detail. In recent years Rovighi * and Herter † have published more extended researches on the toxicity of indol. Rovighi finds that for rabbits the lethal dose of indol or skatol lies between 1.5 and 2 grammes administered in the course of 24 hours by subcutaneous injection, and that these two products of intestinal putrefaction have a similar physiological action. As summarized in Maly's *Jahresbericht*, the symptoms of poisoning are: torpor, somnolence, widespread paresis, weak action of the heart, reduction of temperature and retention of urine and fæces. The autopsy in cases of acute poisoning showed the portal vessels and the supra-hepatic veins to be highly congested, while in cases of chronic poisoning, especially after the administration of indol, the bile ducts were surrounded with infiltrating small cells, which also filled up the intercellular spaces. The kidneys were congested.

Herter's experiments relate to acute indol poisoning in rabbits and dogs, to chronic indol poisoning in rabbits, and to the effects on man of moderate doses taken by the stomach. As in the experiments of Rovighi, it was found that in acute poisoning with considerable quantities, say 70 cc. of a 0.1 per cent solution of indol injected slowly into the femoral vein of a dog weighing 15 lbs., the symptoms were cardiac and respiratory depression, general prostration, irregular clonic spasms, increased reflex excitability and marked contraction of the pupils. The cause of death appeared to be cardiac rather than respiratory failure. Observations on the temperature and on arterial pressure were not made. Of great interest are Herter's experiments on chronic indol poisoning in rabbits. The daily injection of such small quantities of indol as 10 cc. of a 0.1 per cent solution led to death in the course of 13-22 days. Diminished activity, loss of appetite, profound disturbance of nutrition with marked loss of body weight are the points especially emphasized in this connection. A small ring-tailed monkey was found to be far less susceptible than rabbits, for the monkey received 4 cc. of a 0.1 per cent solution daily for two months without any apparent effect. Highly interesting, too, are Herter's contributions to the study of indol poisoning in man. Three healthy men, varying in age from 25 to 32 years, were induced to take indol during periods of from 6 to 13 days in daily quantities varying in the several subjects from 0.025 to 2 grammes. One of these men, a vigorous medical student, aged 25 years and weighing 160 lbs., consumed no less than 6.8 grammes in divided doses in 6 days, taking on one day

* Abstract in Maly's *Jahresb. ü. Thier-Chemie*, xxvi, 456.

† An experimental study of the toxic properties of indol. *New York Med. Journ.*, 1898, July 16 and 23.

as much as 2 grammes. The first day, after a dose of 1 gramme, no symptoms whatever were noted. Further administration with slightly increased doses led to disturbances of sleep and headache but no distinctly toxic symptoms. Without going further into the details of Herter's work, which is of especial value when the clinical significance of indol absorption is to be considered, I will only state that I agree with his conclusion that indol does not ordinarily exert highly toxic effects even when absorbed in unusually large amounts.

My own experiments on indol and skatol relate merely to their effect on arterial pressure. The indol used in my experiments was made according to Nencki's synthesis, acting with dichlor-ether on anilin. I believe that pure indol is more easily secured in this way than from putrefying fibrin. I have found that when injected in doses of 0.1 gramme into the jugular vein of the dog it produces no effect on arterial pressure. In frogs, as pointed out by Christiani,* it produces convulsions similar to those caused by phenol. What has been said of indol holds also for skatol, which has been fed to a dog weighing 55 kilos at the rate of 30 grammes in 21 days without any serious effect.† In my experiments no change of arterial pressure was produced by jugular injections of 0.1 gramme. In fact, I am satisfied that 20 times as much of either of these substances as are excreted daily by a man of 70 kilos weight may be injected at one time into the jugular vein of a dog of 4 kilos without producing an appreciable effect on the circulation or respiration. Indol, however, is much the more important of the two, as skatol, though formed in larger quantities, is absorbed only in traces.‡

When we consider, therefore, the amounts in which any of these substances could probably be formed under the most favorable circumstances, and compare these with the quantities which have been administered empirically or experimentally, we cannot but feel that to account for the symptoms in acute cases of intoxication something more active is necessary.

The third class of substances comprises putrescin, cadaverin and ethylidendiamin, all belonging to the diamins. Udránzsky and Baumann § have fed both putrescin and cadaverin to dogs in large doses without effect. Grawitz || has shown that they are both capable in 2.5 per cent solution of producing severe inflammation and necrosis,

* Loc. cit.

† Mester, *Zeitschr. f. physiol. Chem.*, xii, 130; Brieger, ibid., iv, 414.

‡ Brieger, ibid., i[1], 241.

§ Ibid., xv, 77.

|| Virchow's *Archiv*, cx, 1.

while Behring * has found cadaverin, taken in large doses, poisonous to mice, guinea-pigs and rabbits. The substance found by Kulneff † in a case of gastroptosis is probably ethylidendiamin. It is more poisonous to mice and guinea-pigs than to frogs. In the former it causes lachrymation and salivation followed by violent dyspnœa, lasting until death, which follows in 24 hours or more. So of these substances it may be said that the first two are not extremely toxic and the chemical position of the last is still uncertain.

Of the various toxins which are known to be formed by the action of bacteria, we have not definite knowledge enough to speak until their principles are more completely isolated so that they can be studied as individuals. Of these, however, many are albumoses or of proteid nature and are destroyed according to Nencki by various digestive juices.‡

As the first three classes of these substances differ from what we commonly have in mind when we speak of poisons, so do the symptoms which they are supposed to produce in the so-called autointoxications differ from the toxic picture we see in a case of ileus or acute intestinal obstruction.

We know that the chief symptoms of ileus, such as pain, vomiting, cold clammy sweat, pallid and shrunken features, with possibly subnormal temperature and ultimate complete muscular relaxation, all of which often result in death within one or two days, can be simulated by poisons formed by bacterial activity, and that, too, within a comparatively few hours as, for instance, by the tyrotoxicon of Vaughan.§ Lépine and Molière || have occasionally observed in cases of intestinal occlusion symptoms like those seen in atropin poisoning, namely, dilated pupils and marked redness of the skin, and these authors surmise that death in these instances may be in some degree due to autointoxication from absorption of ptomaines from the intestine.

* *Deutsche med. Wochenschr.*, 1888, No. 24.

† *Berl. klin. Wochenschr.*, 1891, p. 1071.

‡ Ransom (*Deutsche med. Wochenschr.*, 1898, p. 117), however, finds that tetanus toxin passes in large part unchanged through the alimentary canal, its harmlessness when administered by the stomach being due to incapacity of the stomach and intestine to absorb it. Behring (ibid., p. 662) considers that other proteid-like bacterial toxins behave in the same way.

§ *Zeitschr. f. physiol. Chem.*, x, 146.

|| Cited from Eichhorst, Darmstenose, *Real-Encyclop. d. gesamm. Heilk.*, iii edit., v, 430.

It is not my purpose to offer a chemical theory in explanation of any of these various symptoms that arise in the course of an acute and complete obstruction of the intestinal canal at different points in its course. It is my object rather to present a chemical study of the intestinal contents in cases of complete obstruction of the small intestine in order to learn whether other or more powerful poisons than the putrefactive products already isolated can be found under such circumstances. Such poisons if present must exert their action and play their part, be it great or small, in the symptomatology of ileus; certainly the substances so far observed in the intestinal canal are not sufficiently toxic to account for any of the symptoms observed in intestinal obstruction. On the other hand, "shock" and similar expressions are far from giving a rational explanation of the condition described.

When we consider the chemical and physical conditions which exist in a case of this kind we find, first, a closure of the bowel, it may be by hernia, volvulus, intussusception or pressure, but the effect is to convert so much of the digestive tract as may be above the constricted portion into a closed thermostatic tube containing culture materials in the shape of proteids, carbohydrates, etc., kept at body temperature and infected by a varied bacterial flora, air being excluded. In this respect, the conditions are similar to an experiment conducted in the laboratory, where the same materials are used and inoculated with intestinal bacteria, but with this striking difference, that in the former case the tube is composed of animal membrane through which many of the products may pass by absorption, to be taken up later by the portal system and if unchanged in their passage through the intestinal wall (as pointed out before in the case of peptone), perhaps to be oxidized or otherwise changed by the liver cells before reaching the tissues. So that for a chemical theory not only would poisons have to be formed, but in order to produce alarming effects they must be of such a composition that they are not destroyed by the liver, or they must be produced in such quantities that the liver is unable to destroy them as fast as they are absorbed.

For the purpose of this research lecithin was chosen, a substance which is a constituent of all food materials and is widely distributed

in nature. The products formed by its decomposition are not only in some instances of extreme toxicity, but also capable of positive detection and identification. It has been found as a constant accompaniment of cell life, animal and vegetable, but chiefly in brain and nerve tissue, yolk of eggs and the germinating sprouts of plants, to a lesser degree in milk, muscles, etc.

Chemistry and Fate of Lecithin in the Economy.—It has been known for a long time that there are different lecithins according to the fatty-acid radicle contained, but more recently Lippman * found two lecithins in beet residue, one of which gave cholin on decomposition and the other betain; he has therefore suggested that we may have different lecithins depending on the interchangeability of the basic radicle, as we have different lecithins according to the acid radicles present.

We know that these complex molecules split up into different compounds with different arrangement of their component radicles according to the agents employed, but as a result of chemical action and putrefactive processes it has been abundantly shown that lecithin breaks up into glycero-phosphoric acid, fatty acids and basic bodies.

As regards the decomposition and fate in the economy of the different radicles composing the lecithin molecule, Bókai considers it analogous to the fats, and states that lecithin is decomposed during the digestive processes into glycero-phosphoric acid, fatty acids and cholin, and that these products are severally absorbed. According to this view it might be dangerous to consume a great deal of food rich in lecithin (eggs for instance) as cholin is certainly not a harmless substance. Bókai † subjected lecithin to the action of the pancreatic ferments and found that it was split up as above, but he mentions also that bacterial agency was not excluded. From the more recent experiments of P. v. Walther,‡ it seems fair to assume that some lecithin may be absorbed without decomposition, as he always found it present in the chyle of the dog to the extent of from 0.03-0.096 per cent. Hasebroek § has shown in putrefactive experiments, practically anaërobic as he used slime from the river Ill as the source of bacteria, that under these conditions cholin is broken up into methylamin, carbon dioxide and methane.

* *Ber. d. deutsch. chem. Gesellsch.*, xx, 3206. See also E. C. Shorey, *Journ. Amer. Chem. Soc.*, xx, 113.

† *Zeitschr. f. physiol. Chem.*, i, 162.

‡ *Arch. f. Anat. u. Physiol.*, physiol. Abth., 1890, p. 329.

§ Hasebroek, *Zeitschr. f. physiol. Chem.*, xii, 148.

In the case of the lecithin under consideration, which is much the more common form in foods, the basic body contained in its molecule is almost wholly cholin * or trimethyl-oxyethyl-ammonium hydroxide. The composition of this base is

$$N\begin{cases}(CH_3)_3\\CH_2 - CH_2.OH\\OH.\end{cases}$$

It is readily oxidized to a highly poisonous compound isomeric with muscarin and, on losing a molecule of water, a process which may easily occur in the intestine, it yields the almost equally poisonous compound neurin. Neurin is trimethylvinyl-ammonium hydroxide and has the composition,

$$N\begin{cases}(CH_3)_3\\CH = CH_2\\OH.\end{cases}$$

The intimate relation between cholin and neurin is further shown by the fact that, as proved by E. Schmidt, neurin can be changed back to the oxyethyl compound, cholin. It has further been shown by Schmidt † that cholin chloride is decomposed almost entirely by putrefactive action, at 20-30° C., at the end of 14 days yielding large quantities of trimethylamin and a small quantity of a base whose platino-chloride is similar in crystallization and solubility to the neurin salt, and also agrees with neurin in its physiological action. When decomposition was carried on for ten days at 30-33° C. neither cholin nor neurin were present, nor could the presence of trimethylamin be determined with certainty. There can be but little doubt that the crystals isolated by Schmidt from solutions of neutral cholin chloride, which had been infected with hay infusions, consisted in reality of the chloride of neurin, and we must therefore regard the conversion of the relatively non-toxic cholin into the highly poisonous neurin as being within the power of perhaps numerous varieties of bacteria. Hasebroek, as mentioned in connection with lecithin, found on treating cholin solutions with sewage from the Ill, that it was entirely decomposed, yielding methane and carbon dioxide. The solution on treatment with alkali gave an odor of methylamin, and Brieger found that a cholin solution, after the action on it of putrefactive bacteria, gave trimethylamin on treatment with alkali. It will be seen that strikingly different results have occurred from putre-

* Strecker, *Anal. d. Chem. u. Pharm.*, cxxiii, 353.

† *Archiv d. Pharmacie*, ccxxix, 481.

factive experiments on cholin. This, however, is to be expected as the flora in many cases is entirely different, but all observers agree that in the examination of the products of putrefaction whenever cholin is present neurin is also present, although it may be in traces only.

In reference to the toxicity of these substances it has been shown that cholin, previously considered non-toxic, is fairly active, since Gaehtgens * has proved that 0.59 gramme produced almost instantaneous death in a cat. It has been further shown that cholin chloride produces the same muscarin-like symptoms as neurin although the latter are much more intense. Brieger † found that 0.005 gramme of neurin chloride would produce the same symptoms in a rabbit as 0.1 gramme of cholin chloride. He further found that the fatal dose of cholin per kilogramme of rabbit was 0.5 gramme or ten times that of neurin. Boehm ‡ considers the curara-like, paralyzing action of cholin to be like that of artificial muscarin, but the latter is 500 times more toxic.

If therefore we take it for granted that putrefaction takes place in the intestinal canal during obstruction, the toxicity of the substances formed will depend upon the material present and the character of the intestinal flora. It may be that at the time of obstruction the canal is comparatively free from those bacteria which would give rise to toxic substances, or, on the other hand, it may be highly infected.

If the lecithin contained in the food is decomposed in such a way as to give rise to cholin and possibly to neurin we may demonstrate their presence. On the other hand, failure to demonstrate the presence of cholin would not prove positively that the decomposition does not go on in this way; since, as in all experiments on the digestive tract, the substances formed are either further modified or are absorbed so rapidly that it is almost impossible at any one time to obtain a sufficient quantity for positive identification. This difficulty is naturally greatly increased when two or three days are allowed to elapse between feeding and the removal of the intestinal content. The intestinal content is then very small and contains so much bile that it is very difficult to handle. Out of six experiments on dogs in

* *Dorpater med. Zeitschr.*, 1870, i, cited from Boehm, *Arch. f. exp. Pathol. u. Pharmakol.*, xix, 87.

† Ueber Ptomaine, i, 39.

‡ *Arch. f. exp. Pathol. u. Pharmakol.*, xix, 87.

only one was I able to obtain a sufficient amount of a platinum salt for analysis.

It was my intention, in these experiments, to determine whether the lecithin content of the food could give rise to cholin and possibly neurin by decomposition in the intestine in cases of obstruction. The dogs used were therefore fed for two or three days before the operation of closing the intestine was performed on yolk of eggs, which is very rich in lecithin.

The following protocol from my notebook will serve to illustrate the entire series of four experiments:

Expt. 3. Friday, March 29, 2 P. M., anæsthetized dog, male, weight 55 lbs. Placed ligature around intestine just above ileo-cæcal valve. Animal had been fed for three days previously on yolk of eggs. Saturday, March 30, 6 P. M. Animal quiet, does not seem very sick, drinks well but does not eat. Urine of sp. gr. 103.2, acid in reaction, no albumin, strong indican reaction. Sunday, March 31. Dog drinks but does not eat, appears much the same, urine 274 cc., sp. gr. 102.8, reaction acid, no albumin. Indican reaction strong. Monday, April 1. Dog seemed better, but about 2 P. M. managed to tear open the incision in the abdominal wall, and in consequence a loop of the intestine escaped. Dog was killed with chloroform and an autopsy made. Urine for this day up to this time, 160 cc., sp. gr. 103.1, reaction acid, strong indican reaction. It may be said that in no case was there any marked anuria, as the dogs drank freely and did not vomit. As far as the indican reaction was concerned it was strong, but not much more so than I have seen in apparently healthy dogs.

It must be remembered that the indican reactions as usually made cannot be considered quantitative, as the color is produced by oxidation of the indoxyl which cannot be regulated to give quantitative results, as the same agent at the same time produces indigo red and indigo white. I consider Baumann's the best test, namely, equal volumes of urine and strong hydrochloric acid with a few drops of ferric chloride, as there is less chance of over-oxidation by this method.

Autopsy.—Evidences of peritonitis; some excess of peritoneal fluid containing flakes of fibrin, intense venous congestion. This was found to be due to perforation at point of ligature. There were slight adhesions between neighboring intestinal loops. Renal cortex much engorged, papillæ pale, capsule non-adherent. Liver hyperæmic, consistence nor-

mal, gall bladder distended, contents green; adjacent tissues stained yellow. Spleen hyperæmic, veins on surface distended. Heart, veins on surface distended, otherwise normal. Lungs, highly pigmented; some calcareous nodules; otherwise normal. Stomach contents small, reaction acid, whole internal surface hyperæmic, pyloric third stained yellow. Two small ulcerations in cardiac portion about middle of inferior curvature.

Intestinal content small, reaction acid, intensely bile-stained and whole surface hyperæmic. The acid character of the contents continued to within 15 inches of ligature and this lower portion of the ileum was very dark and had apparently lost all tone. It was filled with fæcal material, bright green in color. The most of the mucous surface of the ileum was highly congested and in the lower portion it was easily separable.

In all the experimental cases, except the foregoing, in which there was perforation at point of ligature, the tendency, with ordinary aseptic precautions, is toward recovery. Plastic processes connect the intestinal walls around ligature, necrosis occurs at the point of ligature and a passage is usually established in 5-7 days. This was a source of disappointment in the earlier experiments as, in waiting for the full effects of obstruction in order to obtain as much material as possible, the experiments failed because of the escape of material through newly formed passages, re-establishing the continuity of the intestine. In those animals which were chloroformed in from 70-80 hours after ligature, there was no abnormal appearance of the internal organs, with the exception of the kidneys, in which there was much engorgement of the capsular veins and intense hyperæmia of the cortex, though the papillæ remained pale.

Microscopic Examination.—Kidneys showed infiltration of Bowman's capsule, cloudy swelling of epithelium of convoluted tubules, some necrosis of the epithelium and tube casts.

Chemical Treatment of the Intestinal Contents.—As it was my intention to ascertain by the presence or absence of cholin, whether there had been decomposition of lecithin during the obstruction of the intestine, it was first necessary to choose a method that would totally obviate, if possible, the chemical decomposition of the lecithin in the analytical processes employed. The most suggestive work in this connection is that of Marino Zuco,* who claims that, by the methods of the toxicologists, it is possible to obtain cholin from fresh tissues, blood, etc., and that the

* The so-called Ptomaines in Relation to Toxicological Researches. Abstr. in *Journ. Chem. Soc.*, xlvi, 342.

cholin thus found originates from the splitting up of the lecithins under the influence of the acids and alkalis.

The intestinal contents of the animal described in Experiment 3 were removed with the aid of as little water as possible. Together with the water added the intestinal contents amounted to 280 cc. and had an acid reaction. The whole was treated with four times its volume of absolute alcohol and left with occasional shakings for 48 hours. It was then filtered and being still acid was evaporated in a large dish on the water-bath, the temperature of the fluid not going above 70° C. at any time; absolute alcohol was occasionally added to carry off the balance of water at the same low temperature. When the material had been reduced to a thin syrup it was mixed with a large quantity of powdered glass, evaporated to dryness in vacuo at 45°-50° C. and then placed in a Soxhlet extracter and thoroughly extracted with ether. This removes all of the lecithin, cholesterin and fats, a great deal of coloring matter, extractives, etc. It is of course understood that the method of treatment was governed by the substance sought. If no cholin was present my question could not be answered in the affirmative; on the other hand, the varying statements in reference to the ease or difficulty with which lecithin is decomposed made it imperative that the possibility of its decomposition should be avoided. Marino Zuco is the chief authority for the statement that lecithin is easily decomposed by analytical methods, and the method devised by him includes digesting on the water-bath for 24 hours at 70°. It is apparent therefore that much less injury must result from evaporating the fluid at the same temperature in one-eighth of the time. Further, I find that drying at first and extracting with ether in Soxhlet's apparatus much facilitates succeeding operations.

Schulze and Steiger * claim that in the examinations of certain seed contents made by previous investigators, all the lecithin was not extracted by ether, and they make these deductions from the fact that after shaking the finely ground seeds in a flask with a quantity of ether, allowing it to stand for some hours and then repeating the process two or three times, they were still able to obtain lecithin. This, however, is quite different from 36 hours' extraction in Soxhlet's apparatus, as in my experiment. After extracting with ether for this length of time one may rest assured that every trace of lecithin has been removed.

After the substance had been extracted with ether as described, it was removed, dried, and extracted with absolute alcohol, acidified with hydrochloric acid. Of the more common putrefactive bases only the chlorides

* *Zeitschr. f. physiol. Chem.*, xiii, 365.

of cholin and neurin are soluble in absolute alcohol and also the chlorides of some of the amines. The alcoholic extracts were united and evaporated to a small bulk and were then treated with an alcoholic solution of platinum chloride, the precipitate was thoroughly washed on a filter with alcohol and ether, and was then dissolved off with cold water, in which it proved to be almost entirely soluble. This solution of the platinum chloride double salt was then decomposed with hydrogen sulphide, was boiled and filtered, and a portion of the filtrate was neutralized and tested with the following alkaloidal reagents:

Reagents.	Reaction.
Phosphomolybdic acid	abundant yellow caseous ppt.
Phosphotungstic acid	white ppt. crystalline.
Potassium bismuth iodide	dark brown pulverulent ppt. somewhat sol. in excess.
Potassium cadmium iodide	white ppt. sol. in excess.
Potassium mercuric iodide	yellow crystalline ppt.
Potassium iodide and iodine	dark brown ppt.
Bromine water	reddish ppt.
Mercuric chloride	ppt. white, gradually becoming crystalline.
Gold chloride	yellow granular ppt. sol. on heating.
Platinum chloride	slight cloudiness.
Tannic acid	white finely flocculent ppt.
Picric acid	no precipitate.

The balance of the filtrate was now evaporated down and precipitated with gold chloride and filtered, the gold salt decomposed with H_2S, and the solution boiled and filtered. The filtrate gave the following alkaloidal tests:

Reagents.	Reaction.
Phosphomolybdic acid	abundant yellow ppt.
Phosphotungstic acid	white crystalline ppt.
Potassium bismuth iodide	dark brown pulverulent ppt. somewhat sol. in excess.
Potassium cadmium iodide	slight ppt. sol. in excess.
Potassium mercuric iodide	yellow crystalline ppt.
Potassium iodide and iodine	dark brown ppt.
Bromine water	reddish ppt.
Mercuric chloride	white ppt. gradually becoming crystalline.
Gold chloride	yellow granular ppt.

On the basis of the above reactions the solution was considered to contain only pure cholin chloride, and it was therefore evaporated down, taken up in absolute alcohol and precipitated with alcoholic platinum chloride. The yellow precipitate of double salts was filtered off, washed with alcohol and ether, dried in vacuo at 100° and analyzed.

0.1457 gramme of this salt gave 0.0463 gramme platinum or 31.77 per cent. For cholin: Theory requires 31.64 per cent platinum. Found 31.77 per cent. platinum.

The presence of cholin in the intestinal contents of the animal experimented upon is therefore proven.

On the Presence of Neurin in the Intestinal Contents Examined.—From the fact that there was a precipitate with potassium cadmium iodide, tannic acid and also a slight precipitate with platinum chloride, I considered there was neurin as well as cholin present in my final solutions. Gulewitsch,* in one of the most complete chemical studies of cholin which has yet been published, draws attention particularly to the fact that Brieger and others, working with such solutions and using tannic acid to distinguish qualitatively between cholin and neurin, fell into an error in using this reagent. Cholin chloride, in acid solution, will not give a precipitate with tannic acid, but in neutral solution invariably does so. I have stated previously that neurin has been considered invariably to accompany cholin. It is possible that, using only qualitative tests, an error may have occurred when experimenters did not note whether the solution of chlorides was neutral or acid.

It has already been stated that when the platinum salt of cholin was dissolved on the filter by the free use of cold water a small quantity of a platinum double salt remained undissolved. This salt was, however, found to dissolve in hot water and when the solution had cooled, small, yellow octahedral crystals were deposited which resembled crystals of the corresponding salt of neurin as described by Gulewitch † in his recent paper on neurin and its compounds. Now, these octahedra could not consist of the platinum salt of one of the amines or diamines, for, the former were excluded by testing the original solution from which the cholin and neurin were precipitated by the chlorides of platinum and gold, and the latter were excluded by the fact that their platinum double salts differ in crystalline character from the octahedral crystals here described. On warming gently the original solution with a slight excess of alkali or of a solution of sodium bicarbonate it was not possible to detect the odor of an amine. Unfortunately, this platinum salt, soluble only in hot water, was not obtained in sufficient amount to warrant decomposing it and performing pharmacological tests with it. Nevertheless, I consider the presence of neurin in the intestinal contents, under the experimental conditions set forth in this paper, as almost a certainty.

† *Zeitschr. f. physiol. Chem.*, xxvi, 175.

* *Zeitschr. f. physiol. Chem.*, xxiv, 513.

The following is a tabular statement of the reactions for cholin obtained with the intestinal contents of the dogs used in Experiments 1, 2 and 4, after these contents had been subjected to the chemical treatment already described. In none of these experiments was enough of a salt of cholin obtained to warrant an analysis. The reactions are stated very briefly, but coincide entirely in appearance and character with those given under Exp. 3, and prove that cholin was present in the intestines of all of these animals though in less amount than in those of the animal used in Exp. 3:

Reagent.	Exp. 1.	Exp. 2.	Exp. 4.
Phosphomolybdic acid	ppt.	ppt.	ppt.
Phosphotungstic acid	ppt.	ppt.	ppt.
Pot. bismuth iodide	ppt. brown.	ppt. brown.	ppt. brown.
Pot. cadmium iodide	ppt. white.	ppt. white.	ppt. white.
Pot. mercuric iodide	ppt. yellow.	ppt. yellow.	ppt. yellow.
Pot. iodide and iodine	ppt. brown.	ppt. brown.	ppt. brown.
Bromine	ppt. brown.	ppt. brown .	ppt. brown.
Mercuric chloride	ppt.	ppt.	ppt.
Gold chloride	ppt. yellow.	ppt. yellow.	ppt. yellow.
Platinum chloride		slight cloudiness.	slight cloudiness.
Tannic acid			ppt.
Picric Acid			

The absence of the precipitates with tannic acid in Exps. 1 and 2 were due most probably to the fact that the solution was acid, whereas in 3 and 4 it was neutralized before tests were made.

On the Occurrence of a Ptomaine accompanying the Cholin and Neurin.—In Experiment 1, in which the dog died during the night and was examined the following morning, a ptomaine was found which possessed the following characteristics. Its hydrochloride is very soluble in alcohol and water and crystallizes in fine needles. It gave all the reactions of cholin, but both the platinum and gold salts were quite insoluble in cold water and difficultly soluble in hot water, the gold salt being more easily soluble than the platinum one. The platinum salt, which had been dissolved in hot water and filtered clear into a watch glass, on cooling gave a deposit, which, under the microscope, had the appearance of small, bright yellow spheres which reacted towards light like crystals. An iridescent scum formed on the surface of the water from gradual decomposition of the salt. The gold salt was dissolved in hot water and acidulated with hydrochloric acid, and, as it appeared to be reducing, the liquid solution was quickly filtered and cooled. On cooling it showed a fine yellow granular deposit of spheroidal crystals. On examination the following morning the deposit was quite

dark and a beautiful mirror had been formed on the sides and bottom of the crystallizing dish as well as on the surface of the liquid. Both the platinum and gold salt, therefore, are easily reduced compounds. It is also to be noted that the free base has a penetrating, sweetish odor, and that it is easily oxidized to a brown resin when its solutions are left exposed to the air.

The amount of this ptomaine at my disposal was insufficient for establishing its identity. It agrees in some of its properties, though not in others, with a ptomaine, $C_8H_{13}N$, obtained by Gautier and Étard * from putrefying mackerel and from the decomposing flesh of the ox and horse. It resembles, perhaps, more closely a base, $C_{10}H_{15}N$, which has been isolated from sea-polyps in an advanced stage of putrefaction by de Coninck.† The hydrochloride of de Coninck's base forms fine, deliquescent needles, changes to a brown resin when exposed to the air, and both the platino- and auro-chloride are decomposed by boiling water.

In the intestinal contents of animal No. 4 apparently the same base was present, for, on shaking out the ether from the Soxhlet apparatus with acidulated water a few milligrammes of a gold salt were obtained which resembled the gold salt already described, and on heating, burnt with a smoky, oily flame which gave off a disagreeable odor.

SUMMARY.

My experiments lead me to believe that complete occlusion of the small intestine at its lower end will give rise to the occurrence of cholin, neurin and perhaps other bases, provided the food taken contains any considerable quantity of lecithin. It is not improbable that still other poisons are formed by bacterial action from other constituents of the food in cases of intestinal obstruction. While cholin would have to be absorbed in relatively large amounts to exert a marked toxic action in human beings it is otherwise with neurin, which is many times more intense in its action and must be classed with the exceedingly active poisons. It has been shown both by the experiments of Schmidt and Weiss and also by those recorded in this paper that the poisonous neurin may be formed from cholin by bacteria. In its physiological action neurin agrees closely with muscarin; especially to be noted here is the paralytic action on the heart and its power to

* Vaughan and Novy, Ptomaïns and Leucomaïns, 3rd edit., 316.
† Ibid., 318.

increase the intestinal movements to such an extent that continual evacuations occur. Whether the ptomaine which was found by me is poisonous I cannot yet say. It must be considered proved, however, that highly toxic substances may arise in the intestinal canal during its complete occlusion. The method of treating cases of intestinal obstruction, before surgical means are resorted to, namely, washing out the stomach and as much of the gut as possible often reduces the violent paristalsis and this is due, perhaps, to the removal of substances out of which irritating and toxic products are formed by bacteria.

In conclusion, I would remark that our knowledge of the fate of lecithin in the digestive canal under normal conditions is very deficient. The assumption that it is saponified by the fat-splitting enzyme of the pancreatic juice, thus yielding cholin, glycero-phosphoric acid and fatty acids, rests on the work of Bókai * in 1877 and, as that investigator himself admits, without excluding bacterial action. This omission throws grave doubts on the results. If the assumption of Bókai be correct, caution must be observed in the use of some foods that have been considered most nutritious and healthful; for instance, the ingestion of a meal made up largely of eggs would hardly be without danger because of the poisonous action of the large quantity of cholin liberated from the lecithin and the probability of the formation of the highly poisonous neurin.

It is my purpose in the near future to examine this question with the help of modern methods.

* *Zeitschr. f. physiol. Chem.*, i, 157.

ON THE VALUE OF UROTROPIN AS A URINARY ANTISEPTIC WITH ESPECIAL REFERENCE TO ITS USE IN TYPHOID FEVER.*

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In a previous number of this Journal† the writer published an article "Upon the Presence of the Typhoid Bacillus in the Urine," and, as the result of observations upon 38 cases of typhoid fever, drew the following conclusions: (1) Typhoid bacilli were demonstrated in 9 out of the 38 cases (about 25 per cent). (2) The bacilli, when demonstrated, were always present in large numbers, and in practically pure culture. (3) The bacilli appeared first in the later stages of the disease, and persisted, in the great majority of cases, far into convalescence. The urine of typhoid patients should, therefore, not only be rigorously disinfected during the disease, but should also be carefully supervised during convalescence. (4) The typhoid bacilli were practically always associated with albuminuria and the presence of renal casts. On the other hand, urines containing considerable amounts of albumin and casts in large numbers often showed no typhoid bacilli. (5) Irrigation of the bladder with antiseptic solutions offers a possible means for removing permanently the bacilli from the urine.

Since the publication of that article two further contributions to the subject have come to my notice, the first by Horton-Smith and the second by Petruschky. The work of Horton-Smith appeared in 1897 in the *Transactions of the Medical and Surgical Society* of London, and should properly have been included in the literature mentioned in my previous article. Horton-Smith examined the urines

* Final report of work done under the provisions of the Dalton Scholarship for 1897-8.

† *Journal of Experimental Medicine*, 1898, iii, 349.

of 7 typhoid patients, with 3 positive results. His conclusions were in general very similar to mine. Horton-Smith also pointed out the fact that the typhoid bacilli can be demonstrated oftentimes in cover-glass preparations from the urine; and, further, that the organisms may be so numerous as to render the urine distinctly turbid.

Petruschky * examined the urines of 50 typhoid patients with but three positive results. Petruschky followed the cases into convalescence, and found that the first case retained the bacilli two months after the temperature had reached the normal point; that in the second case the bacilli were still present a month and a half after the disappearance of fever; that in the third case the organisms had disappeared eight days after the beginning of convalescence. The fact that the organisms were present in enormous numbers was strongly emphasized by Petruschky. According to his calculation a single cubic centimetre of urine, in one case, contained 170,000,000 typhoid bacilli.

Finally, through the courtesy of Dr. Harvey Cushing, I am permitted to report briefly a most remarkable case observed recently at the Johns Hopkins Hospital. The patient, a man, had been in the Johns Hopkins Hospital five years before with an attack of typhoid fever. Ever since his discharge from the hospital he had had trouble with his urine. Investigation showed him to be suffering from a cystitis, and the typhoid bacillus was obtained in pure culture from his urine.

We see, therefore, in the light of the accumulated evidence, that our supervision of patients sick with typhoid fever has hitherto been very inadequate; that we must disinfect carefully the urines as well as the stools of typhoid patients; that the necessity for such disinfection and supervision does not cease with the fever, but must be kept up, oftentimes for weeks and sometimes for years.

In the previous article already referred to, the question of treatment for this condition was barely touched upon. In a single case the bladder was irrigated with antiseptic solutions, and the results demonstrated the comparative uselessness, as a remedy, of boric acid, and the effectiveness of corrosive sublimate. Irrigation, however, with its necessary catheterization, is not a method of treatment easily

* *Centralbl. f. Bakt.*, 1898, xxiii, 577.

applicable, and it was next determined to try the effect of the so-called urinary antiseptics, salol, benzoate of ammonia, urotropin, etc., given internally.

The number of cases of typhoid fever comprised in the present series is 66, and the number of specimens of urine examined 190. Of the 66 cases, 14 showed the presence of typhoid bacilli in the urine. The percentage of positive results is therefore somewhat smaller than in the previous series, a difference due possibly to the fact that, in the latter series, the urines were first examined at or about the beginning of convalescence; whereas, in the former, investigation was begun early in the disease. For this reason it is entirely possible that in a number of cases the urines had contained bacilli, but that these had disappeared spontaneously before investigation was begun. Every case showed at some time during the disease a positive typhoid serum reaction (Widal).

The methods employed in obtaining the urines, and the tests applied in the identification of the typhoid cultures, were the same as in the previous series.*

The positive cases, their course and treatment, may be outlined briefly as follows:

Case VIII. Clinical course severe. Relapse. Urine was examined on the 39th, 46th, 50th, 53rd, 57th, and 61st days. Salol was given, 10 grs. t. i. d. from the 42nd to the 55th day. The bacilli persisted, however, in large numbers, and the patient left the hospital with the condition unrelieved.

Case IX. Clinical course very mild. Bacilli present in the urine of 22nd and 28th days. The number of organisms was always small. Treatment: salol 10 grs. t. i. d. from the 26th to the 35th day. Urine of the 32nd and 35th days showed no bacilli.

Case X. Clinical course very severe. Relapse with streptococcus infection. Death. Bacilli present in urine in very large numbers on the 19th, 25th, 43rd, 76th, and 79th days. No treatment was instituted in this case.

Case XVI. Clinical course very severe, followed by cholecystitis. Cholecystotomy was performed and many stones of considerable size evacuated. Fluid from the gall bladder showed typhoid bacilli in pure

* Loc. cit.

culture. In this case salol was given in 10 grain doses three times a day from the 28th to the 35th day. The urine became dark on standing. In spite of the treatment, however, typhoid bacilli were found in large numbers in the urines of the 28th, 35th, 39th, and 43rd days. At the suggestion of Dr. H. F. Vickery it was then resolved to try urotropin, and the drug was given in doses of 10 grains three times daily from the 45th to the 54th day. Further examination of the urine was made on the 47th, 49th, 52nd, 57th, and 71st days, and not a typhoid bacillus could be demonstrated. Moreover, it is to be noted that upon the day of the last examination (71st day) 17 days had elapsed since the last dose of urotropin. The removal of the bacilli was, therefore, permanent, and not confined merely to the time during which the drug was administered.

Case XV. Clinical course moderately severe. Typhoid bacilli were found in large numbers on the 32nd day. Urotropin, 10 grains t. i. d., was given from the 33rd to the 38th day, and from the 45th to the 53rd day. The urines of the 35th, 38th, 45th, 53rd, and 67th days showed no typhoid bacilli. After 50 grains of urotropin the bacilli had disappeared, and two weeks after the administration of the drug had been stopped no bacilli had reappeared.

Case XXV. Clinical course very severe. Death. Typhoid bacilli found in urine of the 15th day. Case not treated.

Case XXVIII. Clinical course moderately severe. Typhoid bacilli in very large numbers found in urines of 34th and 38th days. Urotropin, 10 grains t. i. d., was given from the 38th to the 42nd day. On the 40th day no bacilli could be found—a fact which is the more remarkable when it is noted that the patient vomited four of the five doses given. The amount of urotropin actually absorbed must have been very small, still its action was effective. On the 47th day—5 days after urotropin was stopped—no bacilli had reappeared in the urine, and a similar result was obtained upon the 108th day.

Case XXXIII. Clinical course moderately severe. Typhoid bacilli present in large numbers on the 25th day. Urotropin, 10 grains t. i. d., was given from the 26th to the 27th day. On the 27th day, after only 20 grains had been given, the bacilli had disappeared. Further examination on the 70th day showed no bacilli.

Case XXXIV. Clinical course moderately severe. Bacilli present in urines of the 12th and 13th days in large numbers. Urotropin, 10 grains t. i. d., from the 13th to the 16th day. On the 14th day, after 30 grains of the drug, the bacilli had not entirely disappeared, but the number

had been reduced from millions to hundreds per cubic centimetre. On the 15th day, after 60 grains of urotropin, no bacilli were seen. On the 20th day there was also a negative result. On the 38th day, however, I was much surprised to find that, not only had the bacilli returned to the urine, but there was also a mild cystitis—a condition which had not been present previously. Urotropin was at once resumed, and given from the 38th to the 47th day. On the 40th day no bacilli could be cultivated from the urine, and the evidence of cystitis had largely disappeared. The urines of the 42nd and the 47th day showed no bacilli. On the 50th day, three days after stopping urotropin, the bacilli had again reappeared, though in comparatively small numbers. Urotropin was again begun, this time in 20 grain doses three times daily. After 40 grains no bacilli were found. On the 55th day the drug was again stopped. On the 64th day the bacilli had not reappeared.

Case XLIII. Clinical course severe with relapse. Typhoid bacilli present in moderately large numbers on the 14th, 23rd, and 37th days. Urotropin, 10 grains t. i. d., was given from the 38th to the 40th day. Urine of the 40th day, after 40 grains of the drug, showed no bacilli.

Case XLIV. Clinical course moderately severe. Typhoid bacilli first found on 38th day. Urotropin, 10 grains t. i. d., from the 39th to the 43rd day. Urine examined on 43rd, 50th, and 56th day, but no bacilli were found after 40 grains of urotropin had been taken.

Case LIII. Clinical course very severe. Typhoid bacilli in moderately large numbers on the 61st and 62nd day. On the 64th day urotropin, 10 grains t. i. d., was begun and continued till the 73rd day. On the 65th day (after 40 grains) the bacilli still persisted in small numbers (hundreds to the cubic centimetre). On the 66th day (after 90 grains) no bacilli could be found. On the 68th day (after 150 grains) a cubic centimetre of urine showed two colonies of typhoid bacilli. On the 70th day (after 210 grains) no bacilli were found. Examinations made upon the 78th, 79th, and 92nd days were similarly negative.

Case LV. Clinical course moderately severe. Urine of 21st and 22nd days showed typhoid bacilli in enormous numbers, as well as a cystitis of moderate severity. Urotropin, 10 grains t. i. d., was given from the 22nd to the 31st day. On the 24th and 27th days no bacilli could be demonstrated. Moreover, the evidences of cystitis had decreased markedly. On the 34th day a few bacilli were found, but they disappeared without treatment on the 35th day. The patient now left the hospital, and was instructed to continue the urotropin as a precautionary measure from the 36th to the 43rd day. Further examination on the 50th day showed no bacilli.

Case LVII. Clinical course extremely severe and complicated by a pneumonia. Death. Bacilli first found in urine obtained at autopsy.

To sum up briefly, we find that—

(1) Of 66 cases of typhoid fever, 14 showed the presence of typhoid bacilli in the urine.

(2) Eleven cases were submitted to treatment with the following results:

(*a*) Two cases received salol alone, and in one instance the bacilli disappeared.

(*b*) One case received first salol, with negative results. Urotropin was then substituted for the salol, and the bacilli disappeared almost immediately.

(*c*) Nine cases (including the case treated first with salol) were treated with urotropin, and in every instance the use of the drug was followed by the disappearance of the bacilli. Moreover, this remarkable result was accomplished in eight out of the nine cases by 60 grains or less of the remedy. A single case (LIII) required 200 grains to remove the organisms. Seven cases were followed for 7, 9, 13, 14, 17, 19, 43 and 66 days, respectively, after the administration of urotropin was stopped, and no bacilli had reappeared. It is fair to assume, therefore, that in these cases the removal of the typhoid organisms was permanent. One case was not followed subsequent to the omission of the urotropin.

Aside from the question of treatment, few conclusions different from those stated in the previous article can be drawn.

As already pointed out by Horton-Smith, the bacilli are often so numerous as to cause distinct turbidity in the urine. For this reason a turbid urine (freshly passed), especially if acid in reaction, should always be regarded with suspicion. Moreover, if such a urine when examined microscopically, either fresh or stained as recommended by Horton-Smith, shows, as it often does, the presence of bacilli, it can be prophesied with great probability that the organisms are typhoid bacilli, and the results of the cultures can be foretold with considerable certainty.

THE NATURE AND ACTION OF UROTROPIN.

Urotropin was first introduced in 1894 by Nicolaier,* and is said to be formed by the action of four molecules of ammonia upon six molecules of formaldehyde. It appears in the urine partly as urotropin and partly as formaldehyde. Urine containing urotropin gives with bromine water a yellow-brown precipitate of dibrom-urotropin. It appears in the urine as early as fifteen minutes after its administration, and can be demonstrated twelve hours after a single dose of 7½ grains. Indeed in rare instances the drug can be demonstrated for two weeks after its administration has been stopped.

Nicolaier claimed that urotropin would assist in the solution of uric acid concretions, and, further, would prevent the development of bacteria in the urine.

Casper † and Mendelsohn † could not agree with Nicolaier as to the solvent powers of the drug, but as a urinary antiseptic they recommended it very highly, especially in suppurative pyelitis and cystitis. Casper also found urotropin valuable in so-called essential phosphaturia, and before all operations upon the urinary tract he used the drug to render the urine aseptic. Elliott § praises urotropin highly as a remedy in cystitis, and finds it much superior to salol, benzoate of ammonium, resorcin, napthalin, guaiacol, and boric acid. Elliott observed no ill effects whatever from the use of the drug, and this has been the experience of all other observers. Rarely as the result of large doses there may occur burning sensations at the neck of the bladder, with increased frequency of micturition, but these symptoms disappear immediately upon the omission of the drug or the reduction of its dose.

In my series of typhoid cases a large number had renal disturbance, as evidenced by the presence of albumin and casts in the urine, but in no case did the urotropin increase the gravity of the condition. In one case, as already stated, the drug caused nausea and vomiting, but this effect was due, I am sure, to the fact that, through a mistake,

* *Centralbl. f. d. med. Wiss.*, 1894, p. 897, and *Deutsche med. Woch.*, 1895, p. 541.
† *Deutsche med. Woch.*, 1897, Therap. Beilage, p. 75.
‡ *Berlin. klin. Woch.*, 1898, p. 48.
§ *North American Pract.*, 1897, ix, 451.

the remedy was given on an empty stomach, instead of in the proper manner, after food.

Cohn * reports great improvement in cases of chronic cystitis associated with enlarged prostate and urethral stricture. In cystitis following acute gonorrhœa, and in tubercular cystitis, there was no improvement.

Loebsch † used urotropin in the case of a woman who had large amounts of uric acid in the urine, and who, moreover, had symptoms of stone in the kidney. The use of urotropin was followed by complete recovery.

My own experience with urotropin in conditions other than typhoid fever is limited, but confirms in general the results obtained by others. In a later paper I shall hope to give a detailed account of these cases.

Returning to the question of the urine in typhoid fever, we draw, as the result of these investigations, the following conclusions:

(1) The urine of typhoid patients may contain typhoid bacilli in enormous numbers.

(2) The bacilli may persist in the urine for weeks, months, or even years, and thus constitute (*a*) a danger to the patient himself (cystitis, orchitis, and epididymitis?), and (*b*), what is much more important, a grave source of danger to the public health.

(3) The necessity for the rigid disinfection and supervision of typhoid urine is apparent.

(4) In 9 cases of typhoid fever in which the urine contained typhoid bacilli, urotropin never failed to remove the organisms. In 7 cases the elimination of the bacilli was permanent. In one case the omission of the urotropin was followed upon three separate occasions by the return of the typhoid organisms to the urine. The fourth attempt was successful. One case was not followed after the urotropin was stopped. There is therefore the possibility that in this case also the bacilli returned, but I do not regard this as probable.

(5) In the great majority of cases 60 grains of urotropin are sufficient to remove the typhoid bacilli. Exceptionally, however, as much as 200 to 300 grains are required.

* *Berlin. klin. Woch.*, 1897, p. 914.
† *Wien. klin. Woch.*, 1897, No. 12.

(6) Inasmuch as it is impossible, without bacteriological examination, to tell whether or not a urine contains typhoid bacilli, all typhoid patients should receive urotropin, 30 grains daily for 10 days, beginning with the third or fourth week of the disease, as convalescence approaches. Probably equally good results could be accomplished by the administration of small doses (10 grains daily) of the drug throughout the disease.

I take this opportunity of expressing my thanks to the members of the Visiting and House Staff of the Massachusetts General Hospital for the abundant clinical material placed at my disposal. I am also much indebted to the Director of the Pathological Laboratory, Dr. J. H. Wright, who has assisted me in every possible way in carrying out this work.

REGENERATION OF THE DORSAL ROOT FIBRES OF THE SECOND CERVICAL NERVE WITHIN THE SPINAL CORD.

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INTRODUCTION.

The object of the researches here described was to determine whether there is or is not, within the cord, regeneration of the fibres of the dorsal roots of the spinal nerves following the degeneration caused by injury to the roots between the spinal ganglion and the cord, and also to show the amount of this regeneration, if any should occur.

With this object in view experiments were conducted upon the second cervical nerve in the dog. The selection of this nerve was suggested by the following considerations: In the first place, the posterior root ganglion of the second cervical nerve in the dog lies normally outside the intervertebral foramen, so that experiments upon the roots are much easier and the risk of injuring the cord is much less than it would be were it necessary to open the vertebral canal. Moreover, the second cervical nerve in the dog is connected with the sympathetic system, not by a mixed ramus communicans, but by a gray ramus alone; a fact of some importance, as will be shown later.

Evidences of regeneration were sought for both by physiological and by histological methods. The present article deals with the physiological methods and the results obtained by them, while a discussion of the subject from a histological standpoint will appear later.

The literature of this subject is not extensive, and most of it dates from a period when the ideas concerning the relations of the nerve fibres and cells were less complete than at the present day. In the very brief summary of this literature that is here presented no especial attempt is made to criticise the older results in the light of newer

knowledge, since our object is simply to call attention to the work previously done in this field. On the clinical side, so far as the writers can ascertain, no satisfactory cases are reported for man of regeneration and return of function after lesions involving the destruction of any part of the central nervous system. On the experimental side, the subject of regeneration in the cord or brain has been studied more carefully by histological than by physiological methods. The efforts made to prove by physiological means the possibility of regeneration have been limited mainly to observations upon the return of sensation or voluntary movement. The results are therefore less certain and more open to double interpretation than would be the case if more exact experimental methods had been employed.

H. Müller (1) cut off the tails of lizards and tritons and demonstrated that anatomical and physiological regeneration of the spinal cord in the regenerating tail may take place, although incompletely.

Masius and Vanlair (2) also obtained regeneration and return of function in frogs after excising pieces of the spinal cord. In some cases the regeneration was complete.

Voit (3) claimed a regeneration and partial return of function after removal of the cerebral hemispheres of a pigeon. This claim has probably been disproved by later experimenters, and the fact that in Voit's case the animal never ate voluntarily nor showed signs of fear indicates that the return of function was not more complete than in the cases of Schrader, in which the entire cerebrum was undoubtedly lacking.

Brown-Séquard (4) described a return of function after section of the cord in pigeons and guinea-pigs. In the former the return was complete, in the latter only partial.

Dentan (5), Eichhorst and Naunyn (6) and subsequently Eichhorst (7) described voluntary movements in dogs after section of the spinal cord. But viewed in the light of the work of Schiefferdecker (8) and of the careful description of reflex movements furnished by Freusberg (9), it seems not impossible that these movements were of a reflex character.

In connection with his interesting microscopical work, Kahler (10) made a few experimental observations. He crushed the dorsal roots of the sixth and seventh lumbar and first and second sacral nerves in young dogs. The resulting anæsthesia and loss of reflexes persisted, except that, after a few months, a hard pinch of the toes appeared to cause pain. It is difficult to understand this latter result without supposing that the crushed nerves again formed connections in the spinal cord.

It thus appears that in the lower vertebrates a certain amount of return of function can follow a lesion in the central nervous system, while it is not yet decided certainly whether any such return is possible among higher animals.

METHOD OF INVESTIGATION.

In our experiments we made use of the second cervical nerve and a short description of the anatomical relations of this nerve are given first, as it may make clearer the subsequent account of the results of the experiments.

The dorsal and ventral roots of the second cervical nerve, after uniting in the intervertebral foramen, pass together to the ganglion, which lies about 2 mm. external to the foramen. Beyond the ganglion the nerve trunk divides into a dorsal and a ventral branch. The former in turn divides into three main branches. The ganglion and the origin of these three dorsal branches lie on the ventral surface of the m. rect. cap. (posticus) major, and, as they curve around the lower border of this muscle, these branches begin to diverge from one another. The main branch, n. occip. magnus, has the following cephalad course: Over the ventral surface of the m. complexus, through the substance of this muscle, emerging close to the median line of the neck; thence over the superficial surface of the m. temporalis to terminate in the m. levator auris longior and the integument of the region about the ear. A second branch passes beneath the larger branches of the third cervical nerve, but without communicating with this nerve, and enters the m. complexus, where its finer ramifications anastomose with similar branches of the third cervical nerve. The third branch, after passing beneath the m. complexus, enters the m. splenius, where its ramifications also anastomose with those of the third cervical nerve.

The ventral trunk passes between the m. longus capitis and the m. longissimus capitis, and then internal to the m. sternocleido-mastoideus. It gives off communicating branches to the n. accessorius and divides into the n. auric. magnus and smaller branches, which are distributed to the lower part of the neck.

The method employed in experimenting on this nerve was as follows: The continuity of the dorsal root fibres was destroyed by ligation of the roots between the ganglion and the cord. The animals were allowed to live for a variable period after the operation so that the

lesion might be followed by degeneration of all the nerve fibres entering the cord by the dorsal root, throughout their whole extent central to the point of ligation. After a period of not less than 88 days (the maximum being 151 days), the nerve was tested for return of function. Such a return would be indicated by the reflex variations in blood pressure, pulse rate and respiration, which might follow stimulation of the nerve in question. The nerve and cord were afterwards studied histologically.

As control experiments kymographic tracings were obtained, showing the variations due to stimulation of the second cervical nerve in two normal dogs. In one of these (Dog B) the roots were then ligated in the usual manner and the nerve again stimulated but with negative results. In all cases both the anterior and posterior roots were ligated, as it was impossible to separate them, a fact without significance in the experiment as only motor fibres occur in the anterior root.

Ligation of the roots.—The dog was etherized, the back of its neck shaved, well scrubbed and then washed with bichloride (1:1000). An incision was made about 8 cm. in length, extending from a point about 2 cm. above the protub. occipit. extern. down the mid-line of the neck. This incision passed through the skin and subcutaneous tissue, which were then retracted, exposing the n. occipit. magnus running to the ear. By separating and retracting the muscles and dividing the m. rect. capitis (posticus) major transversely, both the ganglion and the more central part of the nerve were exposed. After carefully freeing the roots from the surrounding tissue, a white silk ligature was passed around them and tied tightly. After a few moments the ligature was removed with a pair of fine, sharp scissors, leaving a constriction of the roots plainly visible at the point of ligation, the two parts of the nerve being united only by a tube of translucent connective tissue—the epineurium—while the continuity of the enclosed fibres was destroyed. The field of operation was then thoroughly irrigated with warm, sterile salt solution, except in the cases of dogs II and III, in which irrigation with bichloride (1:1000) preceded the use of the salt solution; the muscles were brought together with deep catgut sutures and the skin wound was closed with a continuous silk suture; the wound was painted with collodion and dressed with iodoform gauze and bandaged. In none of the cases did suppuration occur.

Test for regeneration.—No antiseptic precautions were observed. The dog was given a hypodermic injection of 0.05 gramme of the sulphate of morphia, and etherized; the trachea and carotid artery were connected with a tambour and a mercury manometer respectively, and these in turn were arranged to record upon a kymographion. A time recorder and a stimulating key also wrote upon the kymographion, so that the kymographic tracings were made to show four simultaneous records, namely, of the circulation, respiration, time, and duration of the stimulation.

The nerve, ganglion and roots were exposed as in the first operation, but greater difficulty was encountered owing to the presence of a considerable amount of scar tissue. In dogs A, I and II, the entire nerve was exposed before stimulating; in dogs B, III, IV, V, VI and VII, stimulations were made as the dissection advanced towards the ganglion.

The electrical stimulations were faradic and of varying strength; weak, moderate and strong. A weak stimulus was a current just perceptible on touching the electrodes to the tongue; a strong stimulus was a current painful to the tongue, and a moderate stimulus was of intermediate strength. Care was taken at each stimulation to prevent radiation through the surrounding tissues. In some cases mechanical stimulation was employed, which consisted in crushing the nerve with forceps, or in tightly ligating it.

After the desired data had been obtained, the animal was killed; the vertebral canal and cranial cavity were laid open with bone forceps; the brain and spinal cord, as far caudally as the sixth cervical nerve, were removed for histological examination. Great care was taken not to injure the brain and cord. The right second cervical nerve with its ganglion and roots was also removed for histological examination.

For the sake of clearness and convenience the results of the kymographic tracings have been tabulated (Table I). The table does not include the results of every stimulation, for where several stimuli in the same region gave identical results only one or two have been taken as examples. Also, all results have been discarded where it seemed possible that they were influenced by any accidents, such as bad electrodes, imperfect isolation of the nerve, etc. The results obtained with the two normal dogs (A and B) are so nearly identical that it has been considered sufficient to insert a single record (Dog A).

EXPLANATION OF TABLE I.

The figures in the column on the extreme left give the order of sequence of the stimuli. The second column states the part of the nerve stimulated. The third column gives the greatest amplitude of the respiratory curve in millimetres: (1) during the period of 10 seconds preceding stimulation, (2) during the period of stimulation, (3) during the period of 10 seconds immediately following the stimulation, and (4) during the succeeding period of 10 seconds. The fourth column gives the rate of respiration per 30 seconds during the same periods of time.

As it is often impossible to appreciate the relative values of small variations in the blood pressure, the approximate *average size* of the pulse waves for the period of 10 seconds preceding stimulation is given in column five. In columns six and seven the blood pressure in millimetres of mercury and the pulse-rate per 30 seconds have been recorded in the same manner and for the same periods as with the respiration. Columns eight, nine and ten state some of the characteristics of the stimuli. Sometimes it was impossible to determine the duration of a mechanical stimulus, and, in such cases, four periods, each of ten seconds duration, were taken in such a manner that stimulation begins with the first second of the second period. Such a procedure is denoted by an asterisk (*) in column ten. In column eleven the results of the stimulations are summed up; "positive" indicating that the stimulation produced reflex changes similar to those obtained with a normal dog; "negative" meaning that there were no appreciable reflexes.

From inspection of the table it appears that a more or less complete return of function occurred in every case, although in dogs IV and VI the reflex effects were very slight. Such an apparent return of function might be accounted for in several ways:

(1) *Imperfect destruction of continuity in the first operation.*—The possibility of this source of error is removed by the following experiments. After obtaining the usual effects of stimulation of the second cervical nerve of a normal dog (Dog B), the roots were ligated in the usual manner. Subsequent stimulation produced no effect whatever. Again in the case of Dog III, after giving the routine stimulations, the roots were ligated in the usual manner. Subsequent stimulations were without effect, and microscopical examination of the nerve showed a complete break in the continuity of the fibres at the point of ligation.

TABLE I.—Dog A. (Normal.)

No.	Part of nerve stimulated.	Respiration. Amplitude.	Respiration. Rate.	Circulation. Amplitude of Pulse wave.	Circulation. Blood pressure.	Circulation. Rate.	Stimulus. Kind.	Stimulus. Strength.	Stimulus. Duration.	Result.
1	*Occipitalis magnus.*	5 41 44 11	7 50 39 22	36	130 145 148 123	47 55 45 44	Electrical.	Moderate.	7 sec.	Positive.
2	Base of skull.	14 47 44 20	16 40 39 35	24	124 148 150 126	52 64 56 48	do.	do.	5 sec.	do.
3	2 mm. from ganglion.	4 34 32 2	6 53 68 11	30	120 150 151 131	36 57 49 37	do.	do.	4 sec.	do.
5		3 13 12 3	10 53 52 32	18	121 140 156 100	52 65 56 48	do.	Strong	7 sec.	do.
					Dog I.					
1	*Occipitalis magnus.*	2 31 32 10	.. 52 44 47	54	136 176 136 120	40 55 48 54	Electrical.	Weak	5.2 sec.	Positive.
2	Top of skull.	3 4 4 ..	31 25 24 ..	38	131 122 125 ..	45 43 43 ..	do.	do.	4.3 sec.	do.
3	Base of skull.	4 13 9 10	24 37 37 29	38	125 140 128 124	42 47 42 44	do.	do.	9.1 sec.	do.
5		10 32 13 10	21 40 48 48	30	122 138 132 118	48 56 49 54	do.	Strong	8.4 sec.	do.
6	15 mm. from ganglion.	7 9 9 9	27 30 27 29	26	131 132 128 127	53 52 47 52	do.	Weak	8.9 sec.	do.
7		8 10 9 6	26 29 28 21	24	126 132 128 126	50 50 48 48	do.	do.	9.3 sec.	do.
8		5 11 10 9	23 30 30 28	30	126 134 128 129	49 50 47 50	do.	Strong	9.1 sec.	do.

TABLE I.—*Continued.* Dog II.

No.	Part of nerve stimulated.	RESPIRATION. Amplitude.				Rate.				CIRCULATION. Amplitude of pulse wave.	Blood pressure.				Pulse rate.				STIMULUS. Kind.	Strength.	Duration.	Result.
13	*Occipitalis magnus.*	0	2	2	2	6	7	7	7	20	130	128	130	132	39	39	38	37	Electrical.	Moderate.	12 sec.	Negative.
14	Base of skull.	2	2	2	2	7	7	6	7	24	128	126	126	127	37	37	36	36	do.	do.	14 sec.	do.
11	3 mm. from ganglion.	0	3	3	2	9	8	6	3	14	106	109	114	117	45	45	45	45	do.	do.	12 sec.	do.
10		3	13	16	10	8	25	34	18	16	122	131	132	110	42	41	45	47	do.	do.	12 sec.	Positive.
17	*Ganglion.*	1	19	19	10	5	31	15	9	20	104	115	116	107	41	41	44	45	do.	do.	8 sec.	do.
18		2	22	22	12	8	39	24	8	16	114	124	106	114	41	42	41	40	do.	do.	8 sec.	do.
16	*Roots.*	2	15	15	6	7	23	12	5	20	116	124	122	114	39	39	40	41	do.	do.	10 sec.	do.
	Dog III.																					
1	*Occipitalis magnus.*	1	1	2	2	6	6	7	7	22	126	121	120	119	59	53	53	52	Electrical.	Weak.	10 sec.	Negative.
2	Top of skull.	3	2	1	2	7	7	7	9	22	120	120	120	123	54	52	51	51	do.	Strong.	18 sec.	do.
19	25 mm. from ganglion.	15	28	..	..	42	47	..	..	7	115	166	142	100	90	84	89	95	Mechanic'l	Crush.	9 sec.	Positive.
21	15 mm. from ganglion.	11	36	30	18	19	44	48	52	7	126	164	110	94	89	..	91	94	do.	do.	8 sec.	do.
6	4 mm. from ganglion.	4	3	4	4	12	12	12	12	18	95	97	97	97	57	60	55	57	Electrical.	Weak.	8 sec.	Negative.
8		4	4	5	5	11	12	12	12	14	103	107	106	106	66	68	69	67	do.	Strong.	7 sec.	.. ?
7		4	4	5	5	11	12	12	12	20	96	97	97	97	59	60	60	62	do.	Weak.	10 sec.	Negative.
9	*Ganglion.*	5	12	12	8	11	23	37	30	14	104	118	124	114	67	76	79	81	do.	Strong.	8 sec.	Positive.
10		4	22	15	4	19	40	36	41	8	118	123	120	102	89	87	87	92	do.	do.	5 sec.	do.

TABLE I.—*Continued.* Dog IV.

No.	Part of nerve stimulated.	RESPIRATION. Amplitude.				RESPIRATION. Rate.				CIRCULATION. Amplitude of Pulse wave.	CIRCULATION. Blood pressure.				CIRCULATION. Pulse rate.				STIMULUS. Kind.	Strength.	Duration.	Result.
1	*Occipitalis magnus.*	1	2	3	3	8	8	12	8	14	98	104	103	97	67	57	56	57	Electrical.	Weak.	5 sec.	Positive.
2		3	2	4	3	8	9	9	11	14	100	105	106	111	53	59	51	51	do.	Strong.	6 sec.	do. ?
3	Base of skull.	2	2	1	1	13	14	10	11	16	104	108	109	106	53	53	49	51	Mechani'l.	Ligation.	10* sec.	do. ?
8	2 mm. from	1	1	1	1	17	17	15	16	7	56	58	58	58	98	96	95	96	Electrical.	Strong.	8 sec.	Negative.
19	ganglion.	1	1	1	1	18	17	18	16	6	63	61	61	60	89	90	90	90	do.	do.	7 sec.	do.
12	*Ganglion.*	1	1	1	1	16	18	19	18	6	58	57	59	61	94	95	93	95	Mechani'l.	Crush.	6 sec.	do.
	Dog V.																					
1	*Occipitalis magnus.*	1	1	1	1	32	32	33	30	38	132	133	132	133	35	35	38	33	Electrical.	Weak.	8 sec.	Negative.
2		1	1	1	1	30	30	30	31	34	133	135	134	134	33	30	36	36	do.	Strong.	9 sec.	do.
3	Top of skull.	2	3	3	3	31	36	48	45	36	136	142	134	122	35	41	48	50	Mechani'l.	Crushing.	10 sec.	Positive.
4		3	8	14	4	45	48	57	52	20	122	142	130	..	50	53	66	..	do.	Ligation.	10* sec.	do.
5		1	1	1	1	26	28	25	24	64	154	155	154	153	31	32	32	30	Electrical.	Weak.	12 sec.	Negative.
6	Base of skull.	1	2	2	1	24	32	36	33	60	154	155	146	144	30	34	36	34	do.	Strong.	10 sec.	Positive.
9		1	1	1	1	23	21	25	28	56	143	140	140	140	30	31	30	30	Mechani'l.	Crush.	11 sec.	Negative.
18	2 mm. from	4	3	4	4	20	22	20	20	5	98	92	96	94	83	80	83	81	Electrical.	Strong.	11 sec.	Positive.
19	ganglion.	2	2	4	4	19	22	19	21	6	91	93	94	98	82	76	79	80	do.	do.	9 sec.	do.
17	*Ganglion.*	3	10	11	4	19	57	18	20	5	100	103	80	86	85	85	84	83	do.	do.	7 sec	do.
20		4	19	11	3	19	62	24	22	5	94	106	84	86	81	79	81	83	do.	do.	6 sec.	do.
25	*Roots.*	2	2	2	2	18	20	19	19	4	94	94	95	95	67	67	68	69	do.	Moderate.	9 sec.	Negative.

TABLE I.—*Continued.* Dog VI.

No.	Part. of nerve stimulated.	RESPIRATION. Amplitude.				RESPIRATION. Rate.				CIRCULATION. Amplitude of pulse wave.	CIRCULATION. Blood pressure.				CIRCULATION. Pulse rate.				STIMULUS. Kind.	STIMULUS. Strength.	STIMULUS. Duration.	Result.
1	*Occipitalis*	2	2	2	2	15	17	17	17	14	118	119	119	119	60	59	59	59	Electrical.	Weak.	18 sec.	Negative.
3	*magnus.*	2	2	2	1	17	17	15	17	14	123	124	122	122	58	58	59	60	do.	Strong.	8 sec.	do.
4	Top of skull.	1	1	1	1	17	18	17	18	14	126	126	128	125	54	59	56	56	Mechani'l.	Crushing.	10 sec.	do.
7	2 mm. from	3	4	3	4	15	15	15	15	8	100	101	98	102	65	65	67	69	Electrical.	Strong.	5 sec.	do.
14	ganglion.	3	4	4	5	19	18	12	18	3	103	99	99	106	101	100	92	92	do.	do.	13 sec.	Positive.
15		4	4	4	3	18	18	19	18	3	107	107	107	107	96	98	93	95	Mechani'l.	Crushing.	10* sec.	Negative.
8	*Ganglion.*	4	4	4	3	15	17	17	15	5	102	97	106	106	78	78	81	71	Electrical.	Weak.	10 sec.	Positive.
13		4	4	4	4	20	20	20	18	3	101	92	100	103	100	98	98	99	do.	Strong.	16 sec.	do.
18	*Roots.*	6	4	6	7	15	12	14	13	2	111	111	106	114	102	90	89	85	do.	Weak.	14 sec.	do.
19		7	4	5	6	20	16	16	15	2	114	103	113	115	90	91	96	84	do.	do.	13 sec.	do.

Dog VII.

1	*Occipitalis*	10	9	7.5	6	30	27	28	25	2	169	169	175	173	75	72	75	76	Electrical.	Weak.	14 sec.	Negative.
2	*magnus.*	7	7	8	6	21	22	23	21	2	172	173	171	173	76	78	73	73	do.	Strong.	14 sec.	do.
3	Base of skull.	6	7	6	..	23	18	18	..	2	173	174	174	..	75	71	70	..	*Mechanical*	*Forceps.*	14 sec.	do.
5	2 cm. from ganglion.	8	14	14	12	12	17	19	18	2	140	152	156	152	93	92.1	93	96	Electrical.	Weak.	14 sec.	Positive.
12	1 cm. from	1	28	16	0	13	54	39	..	1	156	184	118	146	117	..	..	117	do.	do.	47 sec.	do.
7	ganglion.	13	32	46	33	9	74	43	38	1	142	146	144	138	..	..	..	..	do.	do.	9 sec.	do.
10	*Ganglion.*	3	33	38	29	12	70	45	34	1	149	172	122	127	..	..	..	..	do.	do.	12 sec.	do.
11		1	27	23	0	13	71	24	0	1	142	181	112	131	117	..	115	117	do.	do.	7 sec.	do.

(2) *Anastomosis with neighboring nerves.*—The above mentioned experiments on Dogs B and III also show that although the finer ramifications of the second cervical anastomose with the third cervical and with the accessorius, still, it is not possible for impulses to pass from the second cervical nerve to the cord through either of these connections.

(3) *Radiation to the cord through the surrounding tissues.*—This also appears highly improbable, for in every case the nerve was dissected out and retracted from the surrounding tissues before the electrodes were applied; and in the case of the ganglion, where such retraction was impossible, many of the stimulations especially with strong currents were accompanied by control stimulations of the surrounding tissues, and always with negative results.

(4) *Transmission of the impulses through the dorsal root of the nerve stimulated.*—This is considered the only possible explanation for the results obtained. Such a return of function in the dorsal roots can occur only in two ways. The root may be regenerated by fibres having their trophic centres within the cord; or secondly, by fibres having their trophic centres outside the cord. It is the usually accepted view that no sensory fibres are derived from cells within the cord, nevetheless it seems advisable to call attention to the work which goes to prove that the dorsal root of the nerve in question does not contain such fibres. This will be especially useful for reference in the section on histology.

In the chick of four to eleven days' incubation, it has been abundantly proved by von Lenhossék (12), Cajal (13), van Gehuchten (14) and Retzius, that fibres arising from a group of cells situated in the ventral horn, after running dorsally and entering the dorsal root, pass directly through the root ganglion without communicating in any way with the ganglion cells. Kölliker (15) is of the opinion that they are vaso- and viscero-motor fibres on their way to the sympathetic system. Up to the present time, however, no observer has been able to demonstrate microscopically the occurrence of such fibres in the mammalia.

According to the original observations of Waller (16) the trophic centres of all the fibres of the dorsal root are the spinal ganglion cells. Vejas (17) and Joseph (18) stated that they found undegenerated fibres

in the proximal part of sectioned dorsal roots, but no one has been able to confirm their observations. On the contrary, Waller's experiments have been repeated and his results confirmed by Bernard (19), Kahler (20), and more recently by Singer and Münzer (21). Sherrington (22) observed such "undegenerated fibres" in the cat, but he gives excellent reasons for believing that they are "embryonic fibres" (23) and not undegenerated fibres.

It might be claimed that splanchnic efferent fibres occur in the dorsal root (24, 25) of the second cervical nerve, as is probably the case with some of the lumbar and sacral nerves. It has been demonstrated, however, by Gaskell (26) and Langley (27) that the splanchnic efferent fibres traverse the white and not the gray rami communicantes. They have also shown that the second cervical nerve in the dog is connected with the sympathetic system (sup. cerv. gang.), not by a mixed ramus communicans, but by a gray ramus alone (28), the rami viscerales of the upper cervical nerves (as well as of the vagus) being represented by the internal branch of the accessorius.

With regard to the occurrence in the second cervical nerve of trophic fibres to the skin, it may be stated that although ligation was performed on both roots of the nerve in seven dogs, no trophic disturbances resulted in the area supplied by the nerve such as Joseph (29) has described in the cat.

Such fibres, moreover, whether splanchnic-efferent or trophic, would not give reflex effects on stimulation, inasmuch as they would be efferent fibres, and the fact that they probably do not exist is therefore of importance only in relation to the subsequent histological study of the cord and nerve.

Hence it is a justifiable conclusion that this dorsal root contains only such fibres as have their trophic centres situated outside the cord, and consequently the presence of root fibres on the central side of the point at which the continuity of the fibres has been destroyed is proof of a regeneration from the ganglion to the cord, and any return of function is a proof of regeneration of those fibres, both in the root and within the cord itself.

As has been said, a more or less complete regeneration occurred in every case. The character of the regained functional activity varied considerably in the different cases. In some the respiratory effects preponderated, in others the circulatory.

As a commentary to the tables and as a means of calling attention to numerous interesting facts shown by them, the following summary of results is given:

Dog. A (normal). The *amplitude* and *rate of respiration, blood pressure,* and *pulse rate* were in all cases markedly *increased* by stimulation of the second cervical nerve.

Dog I. The *increase* in the *amplitude* of the respiratory curve was as great as in the normal dog (Dog A). The effect on the *rate of respiration* was *variable,* but usually an acceleration was observed. There was a *rise of blood pressure* during the period of stimulation and the *pulse rate* was, in general, *accelerated,* especially during the earlier stimulations.

Dog II. The respiration was but little affected by stimulation of the peripheral nerve, but stimulation of the ganglion and roots caused about as great an *increase* in the *amplitude* as in Dog A, while the *acceleration of rate,* although marked, was proportionally less than in A. Stimulation at the periphery caused very little change in the *blood pressure;* nearer the ganglion a slight *rise,* while at the ganglion and roots the *rise* was pronounced. The *pulse rate* showed little or *no variation.*

Dog III. A *rise* of *blood pressure* occurred with mechanical stimulation of the peripheral nerve and with strong electrical stimulation at and near the ganglion (No. 8), the last being the least marked. Other stimulations caused no variation in blood pressure. The effect on the *pulse rate* was *inconstant.* The *amplitude* and *rate of respiration increased* with the blood pressure, except in case of No. 8, in which no variation occurred.

Dog IV. The *respiration* remained throughout *unaffected.* The *blood pressure* was *slightly raised* by the first three stimulations, after which stimulation produced no effect. The *pulse rate* was altered in the first three cases only. These were stimulations of the peripheral nerve, of which two caused a *slowing* and one an *acceleration* of the pulse rate.

Dog V. The *rate* of respiration was *increased* by mechanical stimulation of the peripheral nerve near its distal end, strong electrical stimulation of the same at the base of the skull, and strong electrical stimulation at the ganglion. In the two last cases the *amplitude,* which elsewhere remained unaffected, was *increased.* The *blood pressure* varied under the same conditions as the rate of respiration, this variation consisting in a *fall* or less frequently a *rise.* The *pulse rate* was affected (*accelerated*) only in the cases of mechanical and strong electrical stimulation of the peripheral nerve.

Dog. VI. The *respiration* remained *unchanged,* except in No. 14. The *blood pressure* was not appreciably influenced by stimulation of the peripheral nerve except in one case (No. 14), but stimulation of the ganglion and roots caused a *fall followed by a rise* to or above the normal. There was a *slowing* of the *pulse* in two cases (Nos. 14 and 18), but otherwise no change occurred.

Dog VII. Stimulation of the more peripheral part of the nerve produced no effect. Stimulation of the nerve near the ganglion, of the ganglion itself and of the roots, caused a marked increase in the *amplitude* and *rate* of respiration. The rise of *blood pressure* was in some cases very marked and usually followed by a fall. The counting of the pulse rate was prevented by the fact that the great oscillations in pressure caused by the violent respirations obscured the pulse record.

The condition of the nerve trunk peripheral to the ganglion was in several instances abnormal, especially in the more peripheral parts of its course. (1) It was in some cases of a dull, opaque, grayish-white appearance (Dogs II, III and VI), and in one case (Dog VI) the ganglion was swollen, elongated and bound down by adhesions. (2) It often responded, not to electrical, but only to mechanical stimuli (Dog III, Nos. 19, 20; Dog V, Nos. 3, 4). (3) It often responded only to the first few stimulations, soon losing its irritability (Dog V, Nos. 3, 4, 9).

All these facts point to a recent regeneration of the peripheral nerve (30). The primary degeneration in the nerve trunk peripheral to the ganglion, which this implies, was considered to be due to the contraction of the scar tissue following the first operation, for this scar tissue, as has been stated, was often quite abundant in the region of the old wound.

When the stimulus was applied to the nerve peripherally to the ganglion, it was often impossible to distinguish between the effects due to recent regeneration of the peripheral nerve and those due to recent regeneration of the root. If, however, a comparison be made of these results with those obtained by stimulating the ganglion or roots, there will be no chance of confusing results due to peripheral with those due to more central regeneration. The condition of the peripheral nerve and of the root, their reaction to stimuli and certain other data of interest and importance are stated in the following table (Table II).

TABLE II.

Dog.	Approximate age.	Intervals between first and second operation.	Scar tissue found at second operation.	Appearance of peripheral nerve.	RESULTS OF STIMULATION.			
					Peripheral.	Nearer ganglion.	Ganglion.	Roots.
		Days.						
I.	Adult.	90	Much.	Normal.	+ e	+ e	..	..
II.	About 6 mo.	92	Much.	Abnormal.	— e	— e	+ e	+ e
III.	About 6 mo.	88	Much.	Abnormal.	+ m, — e	— e	+ e	..
IV.	Adult.	90	Moderate.	Normal.	— m, — e	— m, — e	— e	— e
V.	Young adult.	89	Moderate.	Normal.	+ m, — e	— m (later)	+ e	— e (later)
VI.	About 6 mo.	151	Moderate.	Abnormal.	— m, — e	— m, — e	+ e	+ e
VII.	About 6 mo.	109	Much.	Normal.	— m, — e	+ e	+ e	+ e

e = with electrical stimuli.

m = with mechanical stimuli.

— = negative results.

+ = positive results. For example: + e = positive results with electrical stimuli.

From the results of the experiments described above we feel justified in concluding, that *after severance of the fibres of the dorsal root of the spinal nerves between the ganglion and the cord, regeneration of the fibres into the cord will take place under proper conditions, so that normal reflexes through the respiratory, cardiac and vasomotor centres may be obtained.*

As to the completeness of the regeneration and the average time necessary for the restoration of function, it is not possible for us to speak positively, owing to the small number of our experiments. The results of the seven experiments indicate that there may be great individual differences in the rapidity of regeneration. In some cases the return of functional activity in the dorsal root fibres seemed to be nearly complete at the end of 90 days, while in one case the return was far from complete after an interval of 151 days.

We may add in conclusion that if the posterior root fibres can thus be regenerated in the posterior columns of the cord, there seems reason to hope that the fibres in other tracts may possess the same property, and that therefore it is not impossible that with the proper technique a severed spinal cord might be made to regenerate its broken tracts both ascending and descending.

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ACTION OF FORMALDEHYDE ON ENZYMES AND ON CERTAIN PROTEIDS.

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It is a well known fact that formaldehyde, even in very small amount, will exert a peculiar action on proteid material, hardening it or otherwise altering its chemical and physical properties. Fibrin, when allowed to stand in a solution of formaldehyde even as dilute as 1:500, soon becomes hardened and resists, as will be presently shown, the action of proteolytic enzymes, and although it may finally be digested the process is nevertheless greatly retarded. Again, egg albumen or blood serum to which a small amount of formaldehyde has been added will not coagulate when heated; and numerous other instances might be cited showing the changes that take place when formaldehyde is present.

Formaldehyde also has powerful germicidal properties, and even in small amount will inhibit the growth of micro-organisms. In stronger concentration it will promptly destroy bacteria.

The exact chemical nature of the soluble ferments, or enzymes, is far from being understood. Some authorities claim they are proteid-like substances. When obtained in the purest condition possible they contain nitrogen, and many give some of the proteid reactions. Certain of the purified ferments, however, fail to give the common proteid reactions. When such reactions are given by a ferment presumably pure, it is still impossible to determine whether these are due to contamination with proteid material, or whether they are due to the ferment itself.

The experiments to be described were carried on with a view of determining whether formaldehyde exerts any action on some of the more common soluble ferments, especially those concerned in the processes of digestion. Accepting the common view that enzymes are

proteid compounds, it might be expected that they would undergo alteration in the same way as ordinary proteid substances. Such change in the composition of the enzyme would be shown by a decreased or wholly suppressed ferment action. It should be borne in mind, however, that a decrease in ferment action, or its entire absence, may be due to the action of the formaldehyde on the substance to be digested. Thus, fibrin which has been kept in a solution of formaldehyde 1:100 for a day is scarcely affected by a solution of pepsin-hydrochloric acid, even after being kept at a temperature of about 38° for several hours. On the other hand, formaldehyde may be added to the digestive fluid in the proportion of 1:100, and at the end of several weeks this solution will be found to be still active; in fact, it will dissolve fresh fibrin nearly if not quite as rapidly as a fresh solution of pepsin-hydrochloric acid.

The literature contains but very little concerning the effect of formaldehyde on digestive ferments. According to Loew,* pepsin and disastase lose their activity when left in contact with formaldehyde for one day; the amounts of substances used were 1 gramme of ferment, 10 grammes of water, and 5 cc. of 15 per cent formaldehyde solution (=5 per cent formaldehyde in the mixture). Other ferments, emulsin, papaïn, tryspin, used in the form of crude products, soon give, like many other proteid substances, precipitates with formaldehyde which are very difficultly soluble in acids and in alkalis. Simons † found that formaldehyde has no apparent effect on peptic digestion, but has a very depressing action on the pancreatic ferment (trypsin), even one part in 2000 parts of the solution being sufficient to distinctly retard digestion. We have been able to confirm this statement. Maybery and Goldsmith ‡ tried the action of several antiseptics on peptic digestion. Their results show that the greater the amount of formaldehyde used the greater will be the percentage of fibrin undigested in a given time. In Wurtz's Dictionnaire de Chimie, Deuxième Supplément, vol. iii, p. 313, the action of formaldehyde on proteid material and on ferments is considered very briefly.

* *Journ. f. prakt. Chem.*, 1888, xxxvii, 101.
† *J. Am. Chem. Soc.*, 1897, xix, 744.
‡ *J. Am. Chem. Soc.*, 1897, xix, 889.

The statement is made that the soluble ferments (diastase, pepsin, pancreatin, etc.) are modified completely by formaldehyde, when this is used in solution of sufficient concentration. This statement, as will be presently shown, does not hold true for pepsin, malt diastase and rennin.

In view of the rather incomplete knowledge in regard to the action of formaldehyde on digestive ferments, it was deemed advisable to make a systematic study of the subject. The ferments pepsin, rennin, pancreatin and papaïn were used; these were ordinary commercial preparations, and were found by control experiments to be more or less active. The pancreatin, however, possessed very little diastatic power, and consequently an aqueous extract of fresh pancreatic gland was used for the purpose of observing the effects on the diastatic as well as on the tryptic ferments. The commercial preparations of ptyalin and malt diastase were found to possess but very little activity, and hence saliva and malt were employed instead. Inasmuch as the preparations referred to are far from being pure ferments and contain more or less foreign material, it is obvious that the ratio between the amounts of formaldehyde and ferment cannot be expressed. All that can be given are the amounts of materials used, the dilution, the amount of formaldehyde, the conditions, and the results obtained. In all the tests the conditions most suitable to the normal action of the ferments, *i. e.* temperature, reaction, etc., were adhered to as closely as possible. When ferments having similar properties were compared, the tests were made under conditions as nearly alike as possible. Several series of tests were made with each ferment; the conditions were varied somewhat with each series, as will be seen from the detailed experiments. When doubtful results were obtained, the tests were repeated. In general, the work was carried on in the following manner: A certain amount of the commercial ferment, or of the solution of the ferment, was dissolved in distilled water, and this solution was divided into portions; to each a definite amount of "formalin" was added, so that the liquid contained known amounts of formaldehyde, varying usually from 1:100 to 1:000. These mixtures of formaldehyde and ferment were allowed to stand in corked

flasks at ordinary room temperature. From time to time portions were tested in order to determine whether the activity had been affected, and if so to what extent. The formalin used was approximately a 40 per cent solution, as determined by analysis; it contained only a trace of free acid, too small to be estimated. Fresh, well washed fibrin from the blood of steers was used in testing the proteolytic ferments, and freshly prepared one or two per cent starch paste, made of potato starch, for the diastatic experiments. The experiments were carried on at a temperature of 37°-40°, except in the case of malt diastase, where the temperature of 57°-60° was employed.

ACTION OF FORMALDEHYDE ON FIBRIN.

Benedicenti * gives a review of the work that has been done on the action of formaldehyde on various proteid substances, and also the results of several series of experiments carried on by himself. He confirms the statements that had been made, that formalhehyde hardens proteids and renders them incapable of swelling in dilute hydrochloric acid, and of being digested by pepsin-hydrochloric acid or by pancreatin juice.

Several series of tests were made in order to determine how much formaldehyde must be used and for how long a time it must be allowed to act in order that fibrin would become so altered that it would be incapable of being digested.

Experiment I.—A few small shreds of fibrin were added to portions of 15 cc. each of an active solution of pepsin. Formaldehyde was then added in the proportions of 1:100, 1:250, 1:1000, and 1:2500. The tubes were corked and set aside for 24 hours at ordinary temperature. An equal volume of 0.5 per cent hydrochloric acid was then added to each tube and the tubes were placed in a water-bath maintained at a temperature of 38°-40°, and examined at intervals during the next 24 hours. Table I shows the changes that took place. Tube C served as a control.

In this experiment the fibrin and pepsin were both subjected to the action of the formaldehyde. The fibrin alone was affected.

Experiment II.—Fibrin was allowed to stand in solutions of formaldehyde 1:500 and 1:1000 at ordinary temperature. At the end of three

* *Archiv f. Anat. u. Physiol.* (Physiol. Abtheilung), 1897, p. 219.

days small pieces were removed and squeezed thoroughly, then subjected to the action of a stronger solution of pepsin-hydrochloric acid than that used in the preceding experiment. At the end of three-quarters of an hour the fibrin that had been exposed to the weaker solution of formaldehyde was dissolved almost completely, and disappeared entirely a few minutes later. That which had been exposed to the stronger solution of formaldehyde dissolved more slowly. At the end of an hour it was swollen, and the liquid was slightly cloudy; after another hour it had entirely dissolved.

TABLE I.

MIXTURES OF PEPSIN, FIBRIN AND FORMALDEHYDE—24 HOURS AT ORDINARY TEMPERATURE, THEN DIGESTED AT 38°–40°.

Tube No.	Form-aldehyde.	Examined at the end of			
		1 hour.	2¼ hours.	4 hours.	24 hours.
1.	1:100	No change.	No change.	Fibrin slightly swollen; liquid clear.	Fibrin nearly dissolved.
2.	1:250	Fibrin slightly swollen; liquid clear.	Fibrin dissolving slowly; liquid cloudy.	Fibrin dissolving slowly.	Fibrin nearly dissolved.
3.	1:1000	Fibrin swollen; liquid cloudy.	Fibrin nearly dissolved.	Fibrin dissolved.	
4.	1:2500	Fibrin dissolving; liquid cloudy.	Fibrin almost dissolved.	Fibrin dissolved.	
C.	.....	Fibrin dissolved.			

Similar results were obtained with an aqueous extract of pancreatic gland. The solution used was the one described in Experiment XVIII. Fibrin, which had been exposed to the action of formaldehyde 1:1000 for 24 hours at room temperature, was digested nearly as rapidly as fibrin which had not been exposed to formaldehyde; that which had stood in formaldehyde 1:500 for the same length of time was digested more slowly, though it did not dissolve completely.

Fibrin, which had stood in formaldehyde 1:1000 for 24 hours at ordinary temperature, or for only a few hours at 40°, was digested completely by the commercial pancreatin, though much more slowly than fresh fibrin. When, however, the action of formaldehyde had been continued for a few hours longer at 40°, the pancreatin had no effect.

The results with papaïn will be described later. It might be said here, however, that a strong solution of the ferment had no effect on fibrin that had been exposed to formaldehyde 1:1000 for a few hours at room temperature, or even for half an hour at 40°.

These results show that formaldehyde, even in very small amount, will alter fibrin in the course of a few hours so that it will offer considerable resistance to the action of proteolytic ferments. The action of the formaldehyde is more marked at a temperature of 40° than at ordinary room temperature. Although the fibrin may be digested the process is retarded. Peptic digestion was affected the least.

ACTION OF FORMALDEHYDE ON MILK.

Pottevin* observed that formaldehyde retards the coagulation of milk by rennet, and that rennet and sucrase on contact with strong solutions of formaldehyde become inactive. He also found that inversion of sugar by sucrase or even by acids was retarded or suppressed by formaldehyde. Weigle and Merkel † found that the precipitate of casein in milk containing formaldehyde is coarsely flocculent and voluminous, and that the digestion of milk and egg albumen is prevented by formaldehyde. The action of formaldehyde on the proteids of milk, as well as on other proteid substances, has been studied by numerous other investigators.

Milk is not coagulated by formaldehyde. If formaldehyde is added to milk in the proportion of 1:500, the latter will be altered within a few hours so that it will not be coagulated by an active solution of rennin. If less formaldehyde is added coagulation will take place, but slowly.

The rennet used was Liquid Rennet, made by John Wyeth and Bro. When diluted with four parts of water (1-4), 1 cc. of this dilute rennet added to 10 cc. of fresh milk and kept at 40° would give a solid coagulum in about three-quarters of an hour to an hour. When diluted with an equal volume of water (1-1), 1 cc. would coagulate 10 cc. of milk in about ten minutes, or if the milk were previously warmed to 40°, in two or three minutes.

Experiment III.—Formalin was added to portions of 240 cc. of fresh milk in flasks in the following proportions:

Flask 1 received	24	cc. formalin (40 per cent) =	formaldehyde	1:25
" 2 "	6	"	"	1:100
" 3 "	1.2	"	"	1:500
" 4 "	.6	"	"	1:1000
" C "	no formalin and served as a control. Fresh milk was always used in the control tests.			

* *Ann. Inst. Pasteur*, 1894, viii, 796.

† Forschungsberichte über Lebensmittel. München, 1895, pp. 91-94.

The flasks containing the milk and formaldehyde were corked and set aside in a cool place. At stated intervals portions of 10 cc. were taken out of each flask, 1 cc. of the dilute rennet (1-4) added, and the tubes then placed in the water-bath and kept at 38°-40°. If no coagulation took place within 24 hours they were reported negative. Three sets of tests were made, extending over a period of four days. Table II contains the results.

TABLE II.

Tube No.	Formaldehyde.	Mixtures of Milk and Formaldehyde tested at the end of 4½ hours.	36 hours.	4 days.
1..	1:25	No coagulation.	No coagulation.	No coagulation.
2..	1:100	"	"	"
3..	1:500	"	"	"
4..	1:1000	Prompt coagulation.	Prompt coagulation.	Milk thickened in about two hours and became solid later.
C..	....	"	"	Prompt coagulation.

Experiment IV.—Another series was made, using as before portions of 240 cc. of milk to which formalin had been added in the following proportions:

Flask 1 received 1.2 cc. formalin (40 per cent) = formaldehyde..... ....1:500
" 2 " 0.8 " " 1:750
" 3 " 0.6 " " 1:1000
" 4 " 0.4 " " 1:1500
" C " no formalin and served as a control.

The tests were made in the same way as those just described, using, however, a stronger ferment solution (1-1), which moreover contained formaldehyde in the proportion of 1:500. The same mixture was used for all three series of tests. As will be explained later, the ferment is not affected by very small amounts of formaldehyde, and although the formaldehyde will rapidly alter the casein of the milk, coagulation will result too rapidly for this effect to be of any influence. In the series of tests made on the 4th day the milk was warmed to 40° before use. The results were as follows (Table III):

TABLE III.

Tube.	Formaldehyde.	Mixtures of Milk and Formaldehyde tested at the end of 1 hour.	24 hours.	4 days.
1..	1:500	Thick in 1¼ hours, solid soon after.	No coagulation.	No coagulation.
2..	1:750	Thick in 15 min., solid in 30 min.	Thick in 20 min., solid in 40 min.	No coagulation even after 24 hours.
3..	1:100	Coagulation in about 15 min.	Thick in 20 min., solid in 40 min.	Thick in 1¼ hours, solid soon after.
4..	1:100	Coagulation in 15 min.	Coagulation in 20 min.	Thick in 15 min., solid in 20 min.
C..	....	Coagulation in about 15 min.	Coagulation in 10 min.	Coagulation in 3-4 min.

These results show that milk to which formaldehyde has been added in considerable quantity undergoes alteration. The casein is rendered incapable of being coagulated by rennet ferment. If formalin is added in smaller amount (1:1000 formaldehyde), the casein is acted upon more slowly and will be coagulated slowly or not at all. The action of formaldehyde on rennet ferment will be considered later.

Several tests were made to determine whether the coagula produced by rennet in milk to which formaldehyde had been added would be digested by pepsin-hydrochloric acid. It was found that the casein behaved just as the fibrin had, that is, if very little formaldehyde had been used the process was simply retarded, while if formaldehyde had been added in larger amount digestion would fail to take place.

It is evident from the preceding experiments that a 1 per cent solution of formaldehyde acting on fibrin for 24 hours will render this practically insoluble in pepsin-hydrochloric acid. Furthermore, the addition of formalin to milk alters the casein to such an extent that this will not be precipitated, or but slowly on subsequent addition of rennet ferment.

ACTION OF FORMALDEHYDE ON PEPSIN.

The pepsin employed (Kahlbaum's) was a white powder, very easily and completely soluble in water. One gramme in 1000 cc. of 0.25 per cent hydrochloric acid formed a very active digestive fluid, as will be seen from the rapidity with which fibrin was dissolved. One series of experiments was made in which formaldehyde was added to portions of a solution of pepsin-hydrochloric acid. In another series the formaldehyde was added in the same amounts to like solutions of pepsin in distilled water. In the latter series an equal volume of 0.5 per cent hydrochloric acid was added just before making the tests.

Formaldehyde and Pepsin.

Experiment V.—One gramme of pepsin was dissolved in 1000 cc. of water. 75 cc. of this solution were placed in flasks and additions made as follows:

Flask	1	received	3.75 cc.	formalin (20 per cent) =	formaldehyde	1:100
"	2	"	1.50	"	"	1:250
"	3	"	0.75	"	"	1:500
"	4	"	0.375	"	"	1:1000
"	5	"	0.15	"	"	1:2500
"	C	"	no formalin and served as control.			

After standing at ordinary temperature for 24 hours, 15 cc. of each mixture were measured into a test-tube and an equal volume of 0.5 per cent hydrochloric acid and a few shreds of fibrin were added. The tubes were then placed in a water-bath which was kept at a temperature of 38°-40°. The fibrin in all of the tubes began swelling immediately and at the end of two or three minutes the liquid became cloudy, indicating that digestion was taking place. At the end of half an hour the fibrin in each tube was almost entirely dissolved, and 15 minutes later it disappeared completely. So far as could be observed there was no difference in the appearance of any of the tubes.

The original mixtures were set aside at ordinary room temperature and at the end of two weeks, and again at the end of four weeks, these were tested as outlined above. The results were exactly the same as in the first, *i. e.* the fibrin swelled immediately and disappeared completely in from one-half to three-quarters of an hour. The mixtures of pepsin-formaldehyde all remained clear and contained no deposits, with the exception of the control solution C. This soon began to give indications of decomposition; within three days the solution was cloudy and had a strong putrid odor; yet it was active, since after standing for a month it was able to digest fibrin as rapidly as when perfectly fresh.

In some other work carried on with solutions of pepsin in ordinary tap-water it was found that the ferment lost its activity within 24 hours and was no longer able to digest fibrin. On referring to the literature, however, it was found that certain inorganic salts, which are commonly found in tap-water, exert more or less effect on pepsin, some destroying it completely. The destruction of the ferment action in these cases is thus explained.

Formaldehyde and Pepsin-Hydrochloric Acid.

Experiment VI.—One gramme of pepsin was dissolved in 1000 cc. of 0.25 per cent hydrochloric acid. 75 cc. of this solution were placed in each of six flasks and additions of formaldehyde were made to these as in Experiment V. These mixtures of formaldehyde and ferment were made at the same time as those in the preceding experiment and the tests were made together.

15 cc. of each solution were measured into a test tube and fibrin added. The tubes were then placed in a water-bath and kept at a temperature of 38°-40°. Tests were made at the end of 24 hours, two weeks and four weeks. The results obtained were exactly the same as in the preceding experiment. In fact, if the tubes had not been labeled, it would have been impossible to distinguish one from another.

These results led us to doubt the statement of Loew that formaldehyde destroys the activity of pepsin. Consequently several sets of tests were made, in which the same amounts of ferment and of formaldehyde were used as were employed by him.

Experiment VII.—One gramme of ferment was dissolved in 10 cc. of water and 5 cc. of a 15 per cent solution of formaldehyde were added. In the first set the Kahlbaum pepsin was used, and in the second, a sample made by Parke, Davis & Co., labeled "Pepsin, Aseptic (Pepsin U. S. P. 1890) 1:3000." This was in the form of yellow scales, and was easily and completely soluble in water, and very active. The mixtures of ferment and formaldehyde were then allowed to stand in corked flasks for 24 hours. Inasmuch as formaldehyde in strong concentration rapidly hardens fibrin and renders it incapable of being digested, especially when allowed to act at an elevated temperature, the mixtures of ferment and formaldehyde were strongly diluted with 0.25 per cent hydrochloric acid just before making the test, and small shreds of fibrin were used. The dilutions made in each case were 1:15, 1:30, 1:60. Fibrin was added to portions of each, and the tubes then placed in a water-bath and kept at 40°. The fibrin in each tube was nearly dissolved in about a quarter of an hour and disappeared completely a few minutes later. At the end of five days, and again after three weeks, new portions of the ferment-formaldehyde solutions were diluted and tested as above. The results were exactly the same as those just given.

From this it would seem that Loew did not dilute his formaldehyde-ferment solution, and if so, his failure to obtain digestion would seem to be due, not to the action of formaldehyde on pepsin, but rather to the action of formaldehyde on the fibrin, altering it before the pepsin could act. This view is confirmed by the following experiments:

Experiment VIII.—Mixtures were made using the same amounts of both samples of pepsin, water, and formaldehyde. These were allowed to stand for 24 hours and were then tested as follows: 10 cc. of each mixture were diluted with an equal volume of 0.5 per cent hydrochloric acid, a few shreds of fresh fibrin were added, and the tubes were then kept at 40°. At the same time control tests were made, with small portions of the same mixtures diluted strongly with 0.25 per cent hydrochloric acid. At the end of half an hour, the fibrin in the tubes containing the very dilute mixture was nearly dissolved, whereas that in the other tubes

was dissolving slowly, although the amount of ferment present was much larger. The formaldehyde was evidently hardening the fibrin so rapidly that the pepsin could not accomplish its work completely. At the end of 3 hours there remained some shreds of fibrin still undissolved, and these gave no further evidence of dissolving during the following 6 hours, but appeared hardened.

The same tests were repeated at the end of three days, the mixtures being kept in the incubator for 24 hours in the meantime. The results obtained were exactly the same as in the preceding.

These results fully confirm the statement made by Simons, that formaldehyde has no apparent effect on peptic digestion. These results, taken in conjunction with those obtained in studying the action of formaldehyde on fibrin also confirm the statement made by Maybery and Goldsmith, that with an increase in the amount of formaldehyde there will be a decrease in the amount of fibrin digested in a given time. This, however, is due solely to the action of formaldehyde on fibrin and not to any alteration of the pepsin. In the experiments outlined above small portions of fibrin were used; if, however, larger amounts were employed some of the fibrin would be hardened by the formaldehyde before digestion could take place, and hence the process would be retarded. At the end of a given time there would still be undigested fibrin in the tubes in which formaldehyde was present.

Benedicenti found that proteids, albumin, fibrin, casein, etc., which had lost their power of being digested by gastric or pancreatic juices through the action of formaldehyde, recovered this power after being heated in a current of steam. The formaldehyde was removed from its combination with the proteid. Inasmuch as ammonia rapidly unites with formaldehyde, thus neutralizing its action, it seemed probable that ammonia would act in a manner similar to that of a current of steam, removing the formaldehyde from the proteid and thus restoring to the latter its property of being dissolved by ferments. Several experiments were made in order to determine whether ammonia would have this action on fibrin and on casein previously treated with formaldehyde.

Experiment IX.—Fresh fibrin was allowed to stand in a solution of formaldehyde 1:100 in a closed flask at ordinary temperature. At the end of 6, 24, and 48 hours portions were transferred to dilute ammonia, about 3.5 per cent in some cases, while in other cases very dilute ammonia was used. After allowing the ammonia to act for some time, the fibrin was washed and subjected to the action of pepsin-hydrochloric acid. At the same time portions of fresh fibrin and of formaldehyde-fibrin which had not been subjected to the action of ammonia were also added to pepsin-hydrochloric acid. Table IV shows the results.

TABLE IV.

Tube No.	Time of action of formaldehyde.	Time of action of ammonia.	Results.
1.	.....	.....	Fibrin nearly dissolved in ½ hour; dissolved completely a few minutes later.
2.	6 hours.	.....	Fibrin swollen slightly in 1 hour; liquid clear; nearly dissolved in 5 hours.
3.	"	3.5% NH_3 15 min. 40°.	Fibrin swollen slightly in 1 hour; liquid clear; partially dissolved in 5 hours.
4.	"	3.5% NH_3 30 min. 40°.	Fibrin swollen slightly in 1 hour; liquid clear; nearly dissolved in 5 hours.
5.	.....	.....	Fibrin nearly dissolved in 1 hour.
6.	24 hours.	.....	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
7.	"	3.5% NH_3 5 hours.	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
8.	"	Trace NH_3 5 hours.	No change at the end of 1 hour; fibrin hardened; liquid clear; dissolved in 12 hours.
9.	.....	.....	Fibrin dissolved in about 1 hour.
10.	48 hours.	.....	No change at the end of 6 hours.
11.	24 hours.	3.5% NH_3 24 hours.	Fibrin very slightly swollen; liquid clear at the end of 3 hours; partially dissolved in 6 hours.
12.	"	Trace NH_3 24 hours.	Fibrin very slightly swollen; liquid clear at the end of 3 hours; partially dissolved in 6 hours.

A test made with papaïn gave very similar results. Fibrin was allowed to stand for about 3 hours in formaldehyde 1:100 at 40°. It was then washed and placed in 3.5 per cent ammonia. After allowing the latter to act for 4 hours, the fibrin was washed and added to a strong solution of papaïn, and the tube kept in the incubator over night. On the following day there was no change in the appearance of the fibrin and the ferment solution was perfectly clear, thus showing that no digestion had taken place.

The results with casein agree with those obtained with fibrin. Formaldehyde was added to milk in the proportion of 1:100 and the mix-

ture kept at 40° for half an hour. At the end of that time a portion was removed and to this was added the calculated amount of a 3.5 per cent solution of ammonia necessary to neutralize the formaldehyde present. This mixture was likewise put at 40° for half an hour, then 10 cc. were removed and 1 cc. of rennet solution (1-1) added. No coagulation took place, even after several hours, but a precipitate of coarse floccules appeared.

These results indicate that ammonia will have but little if any effect on the proteid which has been altered by formaldehyde.

ACTION OF FORMALDEHYDE ON RENNET.

The rennet ferment used was the same as that employed in studying the action of formaldehyde on milk. Two series of experiments were made; one with dilute rennet solution (1-4), and the other with stronger rennet solution (1-1). The results were the same in both cases except that with the more dilute ferment solution coagulation took place more slowly. The conditions were the same as in the previous tests on milk, namely, 1 cc. of the ferment solution was added to 10 cc. of milk, and the tubes were then placed in a water-bath and kept at a temperature of 40°. In the absence of formaldehyde coagulation would occur in about three-quarters of an hour to an hour with the dilute ferment solution (1-4), and in about 10-15 minutes with a stronger solution (1-1). If, however, the milk was previously warmed to about 40° coagulation would result much more quickly, sometimes even in one or two minutes.

Formaldehyde and Dilute Rennet.

Experiment X.—Formalin was added in the following proportions to portions of 40 cc. of rennet solution (1-4) in flasks:

Flask	1	received	4	c.c. formalin	(40 per cent) = formaldehyde	1:25
"	2	"	2	"	"	1:50
"	3	"	1	"	"	1:100
"	4	"	0.5	"	"	1:200
"	5	"	0.2	"	"	1:500
"	6	"	0.1	"	"	1:1000
"	C	"	no formalin.	This served as control.		

Tests were made after the mixtures had stood 1½, 4, 7 and 14 days at ordinary room temperature. Table V shows the results obtained. In

the tests made on the 4th, 7th and 14th days the milk was previously warmed to 40° and then the rennet-formaldehyde mixture added.

TABLE V.

Tube No.	Form-aldehyde.	Mixtures of Rennet and Formaldehyde tested at the end of			
		1½ days.	4 days	7 days.	14 days.
1.	1:25	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.
2.	1:50	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.	No coagulation even after 24 hours.
3.	1:100	Coagulation in about 2 hours.	Coagulation in about 2 hours.	Thick in 5 hours, solid later.	No coagulation even after 7 hours.
4.	1:200	Coagulation in about 1¼ hours.	Coagulation in about 1¼ hours.	Coagulation in about 1¼ hours.	Coagulation in about 2½ hours.
5.	1:500	Coagulation in about 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1¼ hours.
6.	1:1000	Coagulation in about 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1 hour.
C.	.....	Coagulation in 40 minutes.	Coagulation in about 1 hour.	Coagulation in about 1 hour.	Coagulation in about 1 hour.

It is evident from this experiment that solutions of rennet containing formaldehyde in strengths of 1:25 and 1:50 will apparently completely lose their ferment action in less than 36 hours. Mixtures containing formaldehyde 1:100 apparently slowly lose their power of coagulating milk, and hence retard the coagulation which, however, still takes place even after an exposure of 14 days. Mixtures containing formaldehyde 1:200 or less practically seem to have no effect on rennet. This loss of ferment action is not, however, due to destruction of rennet but rather, as will be shown later, to the action of formaldehyde on the casein of the milk, rendering this non-coagulable. This action corresponds to that of formaldehyde on egg albumen, on blood serum and on fibrin.

The apparent action of formaldehyde on rennet is well shown in the preceding experiment. This is because the weak rennet solution acts slowly on the milk and hence the formaldehyde has time to act on the casein. When a stronger rennet solution is used, as in Experiment XI, the formaldehyde is not given the time to alter the casein. Hence in Experiment XI coagulation takes place, or is but slightly retarded as compared with Experiment X.

Formaldehyde and Strong Rennet.

Experiment XI.—Formalin was added to portions of 20 cc. of the rennet solution (1-1), in flasks, in the following proportions:

Flask	1	received	1 cc.	of formalin	(40 per cent)=	formaldehyde	1:50
"	2	"	0.5	"	"	"	1:100
"	3	"	0.25	"	"	"	1:200
"	4	"	0.1	"	"	"	1:500
"	C	"	no formalin.	Served as control.			

These mixtures were allowed to stand at ordinary room temperature, and were tested at intervals during a period of 5 weeks. The results are given in Table VI.

TABLE VI.

Tube No.	Formaldehyde.	Mixtures of Rennet and Formaldehyde tested at the end of 1 hour.	24 hours.	4 days.	11 days.	35 days.
1.	1:50	Coagulation in about 1¼ hrs.	Thick in 30 min.; solid in 40 min.	Thick in 1 hr.; solid later.	.....	.....
2.	1:100	.....	Coagulation in 10 min.	Thick in 10 min.; solid in 15 min.	Coagulation in about 30 min.	Coagulation in 15 min.
3.	1:200	.....	Coagulation in 10 min.	Thick in 5 min.; solid in 10 min.	Coagulation in about 30 min.	Coagulation in 10 min.
4.	1:500	.....	Coagulation in 7 min.	Coagulation in 5 min.	Coagulation in 20 min.	Coagulation in 7 min.
C.	.....	Coagulation in 10 min.	Coagulation in 3 min.	Coagulation in 5 min.	Coagulation in 15 min.	Coagulation in 7 min.

From these results it will be seen that formaldehyde, when added to a strong solution of rennet ferment, even in the proportion of 1:50, exerts no apparent effect on the ferment. The mixtures of ferment and formaldehyde were as active at the end of five weeks as when fresh. The slight differences in time required for coagulation by the same solution of ferment on different days are most probably due to differences in the composition or in the temperature of the milk. Market milk was used, and is liable to vary slightly from time to time.

From the tables it will be seen that the ferment solutions which contained the most formaldehyde did not produce a coagulation as rapidly as those in which formaldehyde was present in smaller amount or was entirely absent. This is no doubt due to the rapid alteration which the casein undergoes, especially at a temperature of about 40°, rather than to a change in the ferment. The following tests favor this view.

Experiment XII.—0.25 cc. of 40 per cent formalin was added to 100 cc. of fresh milk, which was already warmed to 40°, making the ratio of formaldehyde 1:1000. This was kept at 40° and portions of 10 cc. were removed and tested at intervals with 1 cc. of rennet solution (1-1).

After an exposure to formaldehyde of 15 minutes the milk would coagulate in 10 to 15 minutes. After standing for an hour, it would thicken in about 25 minutes, but would not become solid till some time later. When tested at the end of two hours the same results were obtained.

On comparison with Experiments III and IV, it will be noticed that when formaldehyde is added to milk in the ratio of 1:1000 there is slow action at ordinary room temperature. After standing for a day or so, rennet will give a coagulation almost as promptly as with fresh milk. If, however, the formaldehyde is added to milk and the mixture kept at about 40°, the action is more rapid, as shown above.

In order to render perfectly clear the action of formaldehyde in Experiments X and XI, attention should be called to the fact that the formaldehyde rennet mixtures were added in portions of 1 cc. to 10 cc. of milk. Consequently the resultant mixture of milk, rennet and ferment contained approximately one-tenth the amount of formaldehyde in the original rennet mixture. Thus, the rennet-formaldehyde mixture (1:50) on addition to milk yields a solution containing formaldehyde 1:500. Now, on reference to Experiments III and IV, it will be seen that the addition of formaldehyde (1:500) to milk altered the casein in 4½ hours and 1 hour respectively to such an extent that in the former case (dilute rennet) no coagulation resulted, whereas in the latter case (strong rennet) coagulation was retarded. It required 1¼ hours to coagulate, whereas in the control test the milk coagulated in 15 minutes. This, it will be seen, corresponds exactly to the behavior of formaldehyde-rennet mixture 1:50 in Experiments X and XI.

If, however, formaldehyde is added to warm milk the action is much more rapid. Portions of 10 cc. each of milk were warmed to 40° and 0.25 cc. of formalin (40 per cent) were then added to each, making the proportion 1:100. After these mixtures had stood for 1, 3, 10 and 15 minutes, 1 cc. of the rennet solution (1-1) was added. Coagulation resulted within 1 or 2 minutes in the first and second tubes, and in about 5 minutes in the third. The milk in the fourth tube did not give any indication of coagulating during the first quarter of an hour. Then it began to thicken, gradually became thicker and finally became almost solid in about an hour.

ACTION OF FORMALDEHYDE ON PAPAIN.

The papaïn used was made by Kahlbaum. It was a very light gray powder, readily soluble in water, forming a clear solution. It was not, however, nearly as active, weight for weight, as the pepsin, and consequently was used in more concentrated solution.

Experiment XIII.—5 grammes of the ferment were dissolved in 400 cc. of distilled water. This solution was divided into portions of 75 cc. each, which were placed in flasks and formaldehyde was added as follows:

Flask 1	received	3.75 cc.	of formalin (20 per cent) =	formaldehyde	1:100
" 2	"	1.50	"	"	1:250
" 3	"	0.75	"	"	1:500
" 4	"	0.375	"	"	1:1000
" C	"	no formalin and served as control.			

After standing for 24 hours at ordinary room temperature, 10 cc. of each mixture were placed in test-tubes; an equal volume of a 0.4 per cent solution of hydrochloric acid and a few small shreds of fibrin were added to each tube. The tubes were then placed in a water-bath and kept at a temperature of 38°-40° for several hours.

Within a few minutes the fibrin in all of the tubes had become swollen, owing to the action of the acid. There was, however, no sign of digestion in any of the tubes in which formaldehyde was present. Even after being kept at that temperature for 24 hours the liquid was perfectly clear and there was no diminution in the amount of the fibrin. Digestion did proceed, however, in tube C, which served as a control. At the end of two hours the liquid was cloudy and about half of the fibrin had dissolved; two or three hours later the fibrin had entirely disappeared.

It did not seem probable that the failure of papaïn to digest fibrin in the above experiment could be due to any action of the formaldehyde on the fibrin. Fibrin, as seen in Experiment II, which has been allowed to stand for three days in a formaldehyde solution of the same strength as that used in mixtures 3 and 4 is quite readily digested by pepsin and by pancreatin. It was found, however, that fibrin which had been exposed to the action of a formaldehyde solution 1:1000 for half an hour at 40° will be digested by papaïn. Moreover, a solution of papaïn in hydrochloric acid, containing formaldehyde in the proportion of 1:1000, will not digest fresh fibrin.

The following experiments will serve to explain the previous results:

Experiment XIV.—1. Some fresh fibrin was added to a solution of formaldehyde 1:1000 and kept at 40°. At the end of 3 and again at the end of 7 hours small portions were taken out, thoroughly squeezed in order to remove the formaldehyde adhering, and then tested with the control solution C used above. Even after standing over night at a temperature of 37°-40° they showed no signs of digesting. The liquid was perfectly clear and there was no diminution in the amount of the fibrin, which, however, had become swollen.

2. Another test was made in order to determine whether fibrin would be altered in less time than three hours so that it would become non-digestible.

5 grammes of papaïn were dissolved in 500 cc. of 0.15 per cent hydrochloric acid, thus making a ferment solution of nearly double the strength of the one used in the preceding experiment. Fibrin was added to a solution of formaldehyde 1:1000 and kept at 40°. At intervals of one-half hour small portions were removed, washed, and added to portions of 15 cc. of the ferment solution, and the tubes kept at 40°. Three tests were made, in which the fibrin had been exposed to the action of the formaldehyde for one-half, one and one and one-half hours, respectively. In none of these did digestion take place. Even after standing in the incubator for 24 hours the fibrin was unchanged in appearance and the liquid was perfectly clear in each tube. In the control tube, however, the liquid became cloudy in a short time, and the fibrin was almost entirely digested within three hours.

3. 1 gramme of papaïn was dissolved in 50 cc. of water and the solution divided into three portions, *a*, *b* and *c;* to *b* formaldehyde was added in the proportion of 1:1000. The solutions were then set aside at ordinary room temperature for 24 hours. Fresh fibrin was then added to *a* and *b*, whereas *c* received fibrin which had stood for 24 hours in formaldehyde 1:1000. An equal volume of 0.3 per cent hydrochloric acid was added to each and the tubes were then placed in the water-bath at 40°. At the end of an hour the liquid in tube *a* was cloudy; the fibrin slowly dissolved during the afternoon. The fibrin in tubes *b* and *c* gave no indications whatever of digesting after being kept at that temperature for several hours. The fibrin was swollen, but there was no diminution in its amount and the liquid was perfectly clear.

4. 5 grammes of papaïn were dissolved in 250 cc. of water and formaldehyde was added in the proportion of 1:1000. The solution was then allowed to stand for 24 hours at ordinary temperature, then divided into two portions. Ammonia was added to the first in sufficient amount to

exactly neutralize the formaldehyde, while double this quantity of ammonia was added to the second. After standing for three hours at ordinary temperature portions of each of these mixtures were placed in tubes, the free ammonia neutralized with dilute hydrochloric acid, and then an equal volume of 0.3 per cent hydrochloric acid and fibrin were added. The tubes were then placed in the incubator at 38°-40° and examined on the following day. The fibrin in both tubes was swollen, but none had dissolved; both solutions were perfectly clear.

The same experiment was repeated after the ammonia had been allowed to act for eight hours. The results were exactly the same as in the preceding.

It was found, however, that ammonia even in very small amount destroyed the ferment. A few drops of a dilute solution of ammonia added to the above solution of papaïn and allowed to act for half an hour rendered it incapable of digesting fibrin.

It is evident from the above results that formaldehyde interferes with papaïn digestion, both by destroying the ferment as well as by hardening the fibrin.

ACTION OF FORMALDEHYDE ON COMMERCIAL PANCREATIN.

Commercial pancreatin (Parke, Davis & Co.) was employed in the following experiments:

Experiment XV.—5 grammes of the yellow powder were dissolved in 400 cc. of water and formaldehyde was added in the same proportions as in the case of papaïn, Experiment XIII, namely, 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. 10 cc. of the control solution, to which an equal volume of one or two per cent Na_2CO_3 solution was added would dissolve a few shreds of fibrin in two or three hours. The proteolytic action was therefore quite strong, though not as marked as that of the aqueous extract of pancreatic gland employed in experiments presently to be described. Inasmuch as this solution possessed very weak diastatic power its action on starch paste was not tried.

After the solutions of ferment and formaldehyde had been made, they were allowed to stand at ordinary temperature for 24 hours. Then 10 cc. were taken from each mixture, diluted with an equal volume of one per cent Na_2CO_3 solution, and small shreds of fibrin added. The tubes

were then placed at a temperature of 38°-40°, and examined occasionally during the next 24 hours. The results are given in Table VII.

TABLE VII.

PANCREATIN-FORMALDEHYDE MIXTURE (24 HOURS OLD), TESTED WITH FIBRIN.

Tube No.	Formaldehyde.	Digested at 38°–40° for 2 hours.	4 hours.	6 hours.	24 hours.
1..	1:100	No change, except that the fibrin seems hardened.	Same as before.	Same as before.	Same as before.
2..	1:250	No change, except that the fibrin seems hardened.	"	"	"
3..	1:500	No change.	"	"	Same as before; fibrin slightly hardened.
4..	1:1000	No change.	"	"	Same as before; fibrin slightly hardened.
C..	....	Fibrin nearly dissolved.	Fibrin entirely dissolved.	"	

There was no diminution in the amount of the fibrin in the first four tubes and no indication whatever that digestion was taking place. It appears from these results that formaldehyde, even in very small amount, prevents pancreatic digestion of proteid material, and that this is due to alteration of the ferment rather than to a change in the material acted upon. It has already been stated under Experiment II that fibrin, when exposed to the action of dilute formaldehyde solution (1:1000) for 24 hours is digested, though the process is retarded. This fact is brought out clearly in Test 3 below.

The following experiment was made in order to demonstrate positively the action of formaldehyde on trypsin. The solution of pancreatin used was the same as that employed in the preceding experiment.

Experiment XVI.—Tube 1 contained pancreatin solution and fibrin, and served as a control.

Tube 2 contained pancreatin and formaldehyde 1:1000 24 hours old, and fibrin.

Tube 3 contained pancreatin to which was added fibrin which had stood in formaldehyde 1:1000 for 24 hours at room temperature.

The results are given in Table VIII.

TABLE VIII.

Tube No.	Digesting mixtures kept at 38°–40°, and examined at the end of 2 hours.	3 hours.	6 hours.
1..	Fibrin dissolving.	Fibrin dissolved.	
2..	No change.	No change.	No change; fibrin is slightly hardened.
3..	"	Fibrin digesting slowly.	Fibrin dissolved.

It is evident therefore that fibrin which had been exposed to the action of a very dilute formaldehyde solution is soon rendered very resistant to the action of commercial pancreatin. The latter, however, is quickly altered and rendered incapable of digesting fibrin.

ACTION OF FORMALDEHYDE ON AN EXTRACT OF PANCREATIC GLAND.

The results with commercial pancreatin were confirmed with an aqueous extract of the fresh pancreatic glands. This extract was also used for the purpose of studying the effect of formaldehyde on the diastatic ferment.

Experiment XVII.—Two fresh glands were cut up as finely as possible and a litre of water was added. The mixture was allowed to stand over night in a cool place. It was then filtered and the clear filtrate was divided into portions, to which formaldehyde was added in the same proportions as in the case of the pancreatin solutions, namely, 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. These mixtures were allowed to stand at ordinary room temperature for 24 hours. Then 10 cc. of each mixture were diluted with an equal volume of two per cent sodium carbonate, a few shreds of fibrin added, and the tubes placed in the water-bath at 38°-40°. The results were as follows (Table IX):

TABLE IX.

PANCREATIC EXTRACT AND FORMALDEHYDE MIXTURES, 24 HOURS OLD

Tube No.	Formaldehyde.	Digested at 38°–40° for 2 hours.	4 hours.	6 hours.	24 hours.
1..	1:100	No change, except that the fibrin seems hardened.	Same as before.	Same as before.	Same as before.
2..	1:250	No change, except that the fibrin seems hardened.	"	"	"
3..	1:500	No change.	"	"	"
4..	1:1000	Fibrin dissolving slowly.	Fibrin about half dissolved.	Fibrin almost entirely dissolved.	
C..	....	Fibrin nearly dissolved.	Fibrin dissolved.		

Only one set of tests was made with these mixtures, since it was found that when formaldehyde had been added to the solution in the proportion of 1:500 or stronger and allowed to act for 24 hours, digestion of fibrin would not take place; and when added in smaller amount, 1:1000, the activity of the ferment was considerably lessened.

Experiment XVIII.—A stronger solution of the trypsin ferment was made than that used in the preceding experiment. Two beef glands, finely divided, were suspended in 500 cc. of water. The mixture was allowed to stand 24 hours and was then filtered. Formaldehyde was added to portions of the clear filtrate in the ratio of 1:500 and 1:1000, while another portion received none and served as a control solution. The solutions were allowed to stand for 24 hours longer. Then 10 cc. of each mixture were diluted with 10 cc. of a two per cent sodium carbonate solution and tested with fresh fibrin, and also with fibrin that had been exposed to the action of formaldehyde 1:500 and 1:1000 for 24 hours. The results are given in Table X.

TABLE X.

MIXTURES OF TRYPSIN AND FIBRIN-FORMALDEHYDE DIGESTED AT 38°–40°.

24 hours' Mixture of trypsin-formaldehyde.	Material used.	Examined at the end of	
		½ hour.	1½ hours.
Formaldehyde, 1:500.	Fibrin.	No change.	Fibrin nearly dissolved.
	Fibrin-formaldehyde, 1:500.	No change.	Fibrin dissolving slowly.
	Fibrin-formaldehyde, 1:1000.	No change.	Fibrin dissolved.
Formaldehyde, 1:1000.	Fibrin.	Fibrin dissolving slowly.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:500.	No change.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:1000.	Fibrin nearly dissolved.	Fibrin dissolved.
Plain trypsin.	Fibrin.	Fibrin nearly dissolved.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:500.	Fibrin dissolving slowly.	Fibrin dissolved.
	Fibrin-formaldehyde, 1:1000.	Fibrin nearly dissolved.	Fibrin dissolved.

At the end of six hours the solutions in the last three tubes had a strong, putrid odor, whereas none of the others possessed any such odor.

When formaldehyde is added in rather strong concentration (1:100 or stronger) to an aqueous extract of the pancreatic gland, rich in ferment and proteid matter, a coarse, voluminous precipitate is thrown down. It was thought that possibly the ferment might be enclosed within this precipitate and prevented from exerting its action through mechanical

interference, although it might still possess its proteolytic property. This was not found to be the case, however, as will be seen from the following experiment.

Experiment XIX.—Two fresh glands were crushed in a mortar with fragments of glass, 250 cc. of water were added, and the mixture allowed to stand over night. It was then filtered, and formaldehyde was added to portions of the clear filtrate in the proportion of 1:25, 1:100, 1:500 and 1:1000; another portion was reserved for control tests. These mixtures were again allowed to stand over night. At the end of that time the first two flasks contained a coarse precipitate, while the other three mixtures were almost perfectly clear. Portions from the first and second flasks were ground finely in a mortar with fragments of glass, an equal volume of one per cent sodium carbonate added, and then small portions of fibrin. The tubes were then placed in an incubator and kept at a temperature of 35°-40° over night. At the end of 24 hours the fibrin in these two tubes gave no indication whatever of dissolving; on the contrary, it appeared hardened. The fibrin in the control tube, however, was completely dissolved within a few hours and the solution had the strong, putrid odor characteristic of tryptic digestion.

Inasmuch as fibrin is rapidly hardened by formaldehyde, especially when the latter is allowed to act at a temperature of 35°-40°, it seemed possible that failure to undergo digestion by a solution of trypsin containing formaldehyde might be made due to this cause. Consequently portions of each of the mixtures of ferment mentioned above were diluted with 20 parts of 0.5 per cent sodium carbonate solution, fibrin was added, and the tubes were placed in the incubator over night. At the end of 24 hours the fibrin in the first tube was slightly hardened, but had not dissolved. The fibrin in the second, third, and fourth tubes had apparently undergone no change whatever; that in the fifth, or control, tube was dissolving slowly. A few hours later the fibrin in the fourth tube was nearly dissolved, and that in the control was completely dissolved.

The following experiment was made in order to determine whether neutralization of the formaldehyde with ammonia would restore the proteolytic property to the ferment.

Experiment XX.—An extract was made as in the above experiments, using two glands and 500 cc. of water. Formaldehyde was added in the proportions of 1:25, 1:100, 1:1000, while another portion was reserved for control experiments. After allowing these mixtures to stand over night, the calculated amounts of ammonia required to neutralize the

formaldehyde were added to portions of each of the mixtures, and they were then allowed to stand for five hours. An equal volume of one per cent sodium carbonate solution and a few shreds of fibrin were added, and the tubes were then placed in the incubator over night. Next morning the fibrin in the first and second tubes had undergone no change; that in the third tube had shown no indications of dissolving during the first seven or eight hours, but later it did dissolve completely. The fibrin in the control tube dissolved completely within about three hours.

A few drops of ammonia were added to another portion of the control solution of ferment, allowed to act for five hours, and then the solution was tested as above. The fibrin in this tube was dissolved with about the same rapidity as that in the control test, showing that a small amount of free ammonia has but very little if any action on trypsin.

The conclusions that are to be drawn from these experiments are that formaldehyde exerts a powerful action on trypsin. When present even in so small an amount as 1:1000, digestion of fibrin is greatly retarded. When added to a strong extract of the ferment in the proportion of 1:500, digestion of fibrin will take place but very slowly. If, however, formaldehyde is added in the same ratio to a weaker solution of the ferment, digestion will fail to take place. That formaldehyde alters the trypsin and renders it inactive is seen from the results in Experiment XIX; for if failure to digest fibrin were due to the hardening action of formaldehyde alone, dilution of the ferment-formaldehyde mixture should make the proportion of formaldehyde so small that its action would be imperceptible, in which case the ferment should dissolve the fibrin. The addition of ammonia will not restore to the ferment its proteolytic property.

ACTION OF FORMALDEHYDE ON AMYLOPSIN.

The influence of formaldehyde on the diastatic ferment of the pancreatic gland was tested in connection with the experiments on trypsin just described. In the first few tests, a starch paste made from corn starch was employed; this was not suited for experiments with diastatic ferments, however, since corn starch is not so easily and rapidly digested as potato starch. Consequently the latter was used instead in the later tests, and only these tests will be given.

Experiment XXI.—The mixtures of extract and formaldehyde employed were those described in Experiment XIX. After these mixtures had stood for about 24 hours, 10 cc. of each were diluted with 10 cc. of a one per cent sodium carbonate solution, and 20 cc. of a freshly prepared one per cent starch paste were added. Two other tubes were prepared, using the formalin-ferment mixtures Nos. 1 and 2 (containing formaldehyde 1:25 and 1:100 respectively), which had previously been ground up finely with fragments of glass. The tubes were kept in the incubator and small portions of each were tested from time to time with iodine solution. The results are given in Table XI.

TABLE XI.

PANCREATIC SUSPENSION AND FORMALDEHYDE (KEPT FOR 24 HOURS AT ORDINARY TEMPERATURE), THEN STARCH ADDED AND RESULTANT MIXTURE KEPT AT 38°–40° AND TESTED WITH IODINE SOLUTION.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 24 hours.
1. (ground.)	1:25	" " " "
2.	1:100	Violet in 15 min.; color gradually became lighter, pink at the end of 24 hours.
2. (ground.)	1:100	Deep blue at the end of 24 hours.
3.	1:500	Pink in 15 min.; colorless in 30 min.
4.	1:1000	Colorless in 15 min. or less.
C.	....	" " "

Experiment XXII.—The mixtures of ferment and formaldehyde were those described in Experiment XX. The tests were made in the same way as in the preceding experiment, and the results are given in Table XII.

TABLE XII.

MIXTURES OF FERMENT-FORMALDEHYDE (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 2 hours; deep violet in 3 hours.
2.	1:100	Violet at the end of 15 min.; colorless at the end 30 min.
3.	1:1000	Colorless in 15 min., or less.
C.	.....	" " "

Experiment XXIII.—Portions of the same mixtures as those used in Experiment XXII were treated with the calculated amounts of ammonia necessary to neutralize the formaldehyde present, allowed to stand at ordinary temperature for five hours, and then tested in the same manner as above. A few drops of ammonia were also added to a portion of the control mixture at the same time. The results were quite similar

to those given in Experiment XXII, although the conversion was slower in each case.

TABLE XIII.

MIXTURES OF FERMENT-FORMALDEHYDE-AMMONIA (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

No. of Mixture.	Formaldehyde.	Results.
1.	1:25	Deep blue at the end of 24 hours.
2.	1:100	Deep blue at the end of one hour; color gradually became lighter; pink at the end of 24 hours.
3.	1:1000	Colorless in about 1 hour.
C.	.....	Colorless in about 30 minutes.

These results show that ammonia will not remove the formaldehyde that is held in combination by the ferment, and thus restore to the latter its amylolytic property. On the contrary, the presence of free ammonia seems to hinder the action of the ferment. This view is confirmed by the following test, made with mixture No. 3 (containing formaldehyde 1:1000).

To two portions of 10 cc. each of this solution 1 and 4 cc. respectively of a 3.5 per cent solution of ammonia were added, and the mixtures allowed to stand for about three hours. An equal volume of starch paste was then added to each and the tubes were placed in the incubator over night. Next morning small portions of each were tested with iodine, after neutralizing the free ammonia with dilute acetic acid. In one case a deep wine color was produced, while in another, in which ammonia had been added in larger amount, a deep violet color was produced. A control test made with the same mixture of ferment and formaldehyde gave no color with iodine after 15 minutes at 40°.

From the above results it will be seen that formaldehyde has a depressing action on the diastatic ferment of the pancreas, though this action is not so marked as in the case of trypsin. Formaldehyde added to an active solution of trypsin in the proportion of 1:500 will completely destroy the action of the ferment, unless the solution is an exceedingly active one. Even when present in so small an amount as 1:1000 the action of the ferment is greatly retarded. On the other hand, formaldehyde added to a solution of amylopsin in the proportion of 1:500 exerts but little action on the ferment after being allowed to act for 24 hours. When present in the ratio of 1:100 conversion of starch is greatly retarded, but will still take place.

Like trypsin, amylopsin seems to be destroyed, and not rendered inactive merely by mechanical interference; and like trypsin, papaïn and saliva, its properties are not restored by adding the calculated amount of ammonia required to neutralize the formaldehyde.

ACTION OF FORMALDEHYDE ON PTYALIN.

Several samples of ptyalin were tested, but inasmuch as these possessed little or no action, saliva was employed instead. The experiments were carried on in general in the same manner as those with amylopsin, and the results were very similar to those obtained with that ferment; they agree also with the results obtained with malt diastase to be described.

When formaldehyde was added to saliva in small amount it had very little effect on ptyalin; if added in larger amount, and the mixture kept at ordinary temperature, it had a depressing action, but did not completely destroy the ferment for several days. If such a mixture were kept at about 40° for some time, however, the action of the formaldehyde was more marked, and the ferment was eventually destroyed.

Experiment XXIV.—Fresh saliva was collected, filtered, and divided into portions of 20 cc. each. Formaldehyde was added to these in the proportions of 1:100, 1:250 and 1:1000; another portion was reserved for control tests. These mixtures were allowed to stand in corked flasks for 24 hours at ordinary temperature. At the end of that time 1 cc. of each was added to 25 cc. of freshly prepared one per cent potato starch paste, the tubes placed in the water-bath at 40°, and at intervals portions from each tube were tested with iodine solution. The results are given in Table XIV.

TABLE XIV.

MIXTURES OF SALIVA-FORMALDEHYDE (24 HOURS OLD) AND STARCH TESTED WITH IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Color gradually changed to light wine-red at the end of 5 hours; colorless next morning.
2.	1:250	Light wine-red at the end of ½ hour; colorless in ¾ hour.
3.	1:1000	Nearly colorless in 10 min.; colorless in less than 20 min.
C.	.....	Colorless in less than 5 min.

The tests were repeated on the following day with almost exactly the same results. Conversion, however, took place slightly more slowly in tubes Nos. 1, 2 and 3. At the same time a series of tests was made in like manner, using portions of these same saliva-formaldehyde mixtures which had been kept in the incubator at about 40° for 24 hours. Conversion took place much more slowly in these, showing that the formalin exerts more effect on the ferment if allowed to act on it for some time at an elevated temperature. The results are given in Table XV.

TABLE XV.

MIXTURES OF SALIVA-FORMALDEHYDE (24 HOURS AT 40°) AND STARCH TESTED WITH IODINE.

Tube No.	Formaldehyde.	Results.
1.	1:100	Deep wine-red at end of 7 hours.
2.	1:250	Light " " "
3.	1:1000	Colorless in about 1½ hours.
C.	.....	Colorless in less than 5 min.

These results were confirmed in the following experiment:

Experiment XXV.—Saliva-formaldehyde mixtures were made in the same manner as in the preceding experiment. Each was divided into two portions, one of these being kept at ordinary temperature, and the other at 35°-40° during the time the experiment was carried on. Tests were made at the end of 4 hours, 24 hours, 3 days and 9 days. 25 cc. of freshly prepared starch paste and 1 cc. of the saliva-formaldehyde mixture were used in each case. The results are given in Tables XVI and XVII; the former table is with the mixtures that had been allowed to stand at ordinary temperature, the latter with those kept at 35°-40°.

TABLE XVI.

Mixtures of Saliva–Formaldehyde (ordinary temperature) and Starch tested with Iodine at the end of

Tube No.	Formaldehyde.	4 hours.	24 hours.	3 days.	9 days.
1.	1:100	Colorless in about ½ hour.	Colorless in about 6 hours.	Light violet at the end of 20 hours.	Deep violet at the end of 24 hours.
2.	1:250	Colorless in about 20 minutes.	Colorless in about ¾ hour.	Nearly colorless at the end of 10 hours.	Light wine-red at the end of 24 hours.
3.	1:1000	Colorless in less than 10 minutes.	Colorless in about 15 minutes.	Colorless in about 4 hours.	Colorless in about 12 hours.
C.	.....	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.

TABLE XVII.

Mixtures of Saliva–Formaldehyde (kept at 35°–40°) and Starch tested with Iodine at the end of

Tube No.	Formaldehyde.	4 hours.	24 hours.	3 days.	9 days.
1.	1:100	Wine-red at the end of 2 hours. Colorless later.	Deep violet at the end of 20 hours.	Deep blue at the end of 20 hours.	Deep blue at the end of 24 hours.
2.	1:250	Colorless in about ¾ hour.	Nearly colorless at the end of 20 hours.	Violet at the end of 20 hours.	Deep blue at the end of 24 hours.
3.	1:1000	Colorless in less than 10 minutes.	Nearly colorless at the end of 1 hour.	Wine-red at the end of 10 hours; colorless next morning.	Deep violet at the end of 24 hours.
C.	.....	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.	Colorless in less than 10 minutes.

Experiment XXVI.—A third short series was made in the following manner: Formaldehyde was added to each of two portions of 20 cc. of saliva in the proportions of 1:100; one of these mixtures was placed in a corked test-tube and kept in the incubator for five days at a temperature varying from 30°-40°, while the other was kept at ordinary room temperature. At the end of five days tests were made as in the preceding experiments. The saliva-formaldehyde mixture that had stood at ordinary temperature gave slow conversion; when tested at the end of ten hours with iodine solution a slight red color was given. The other mixture gave no conversion at all; at the end of 24 hours a deep blue color was produced with iodine. A control test made at the same time with some of the same saliva gave conversion in less than ten minutes.

Attempts were made to remove the formaldehyde from its combination with the ferment by means of ammonia. As in the case of papaïn, trypsin, and amylopsin, these attempts were unsuccessful. Ammonia was added to a portion of a saliva-formaldehyde mixture (formaldehyde 1:500) several days old and the tube was set aside for three hours. The free ammonia was then carefully neutralized with very dilute acetic acid, and the saliva was then added to starch paste. A control test was also made with the same mixture of saliva and formaldehyde. Conversion took place slowly in both, but with the same rapidity; when tested with iodine solution the same colors were produced by both mixtures.

That the formaldehyde acts on the ferment and not on the starch is proven by the following test: Formaldehyde was added to a fresh one per cent starch paste in the proportion of 1:100, the tube corked, and

kept at 35°–40° for five days. Saliva was then added, as in the preceding tests. Complete conversion resulted in less than ten minutes.

It is evident from these experiments that formaldehyde in very small amount exerts but very little action on ptyalin, unless the mixture is allowed to stand for several days or is kept at a temperature slightly above ordinary room temperature for a few hours. When present in larger amount the action is more marked. The ferment is slowly destroyed at ordinary temperature, though it does not completely lose its power even when exposed to the formaldehyde for several days. If, however, the formaldehyde-saliva mixture is kept at an elevated temperature the ferment is destroyed much more rapidly.

ACTION OF FORMALDEHYDE ON MALT DIASTASE.

A water extract of ground malt was used, and also malt itself. The results were exactly the same in both cases. In Experiments XXVII, XXVIII and XXIX starch paste made from corn starch was used, whereas in the remaining experiments potato starch was used.

Experiment XXVII.—100 grammes of ground malt were added to 1000 cc. of water and the mixture allowed to stand for about three hours with occasional shaking. It was then filtered clear, divided into portions, and formaldehyde added in the proportions of 1:100, 1:250, 1:1000 and 1:2000; another portion was reserved for control tests. These solutions were then tested after standing for two and for eight days. 5 cc. of each mixture were added to 25 cc. of fresh one per cent starch paste (corn), and the mixtures were kept at 58°-60°. From time to time small portions of each mixture were tested with iodine solution. The results are given in Table XVIII.

TABLE XVIII.

Mixtures of Malt Extract and Formaldehyde Tested with Starch and Iodine after standing for

Tube No.	Formaldehyde.	2 days.	8 days.
1.	1:100	Light violet at the end of 2 hours.	Light violet at the end of 4 hours.
2.	1:250	Colorless in about ¾ hour.	Light violet in 30 min.; colorless in about ¾ hour.
3.	1:1000	" " "	Pink in 30 min.; colorless in about ¾ hour.
4.	1:2000	" " "	Pink in 30 min.; colorless in about ¾ hour.
C.	.....	Blue at the end of 2 hours.	Blue at the end of 4 hours.

A duplicate set of solutions made in the same way and at the same time gave exactly the same results.

Experiment XXVIII.—Another set of solutions was made in the same manner as above and formaldehyde was added in the proportions of 1:100, 1:500 and 1:1000. These were tested with two per cent and with one per cent starch paste (corn) after standing for 24 hours. In this series 1 cc. of the ferment solution was added to 20 cc. of the starch.

TABLE XIX.

MIXTURES OF MALT EXTRACT AND FORMALDEHYDE TESTED WITH STARCH PASTE AND IODINE, AFTER STANDING 24 HOURS.

Tube No.	Formaldehyde.	2 per cent starch.	1 per cent starch.
1.	1:100	Deep violet at the end of 4 hours.	Light violet at the end of 3 hours.
2.	1:500	Light violet at the end of 4 hours.	Colorless in 1 hour.
3.	1:1000	Colorless in about 3 hours.	Colorless in 1 hour.
C.	.....	Nearly colorless in 5 hours.	Light violet at the end of 2 hours.

Experiment XXIX.—Ten grammes of malt and 100 cc. of water were placed in each of half a dozen flasks, and formaldehyde was added in the proportions of 1:100, 1:250, 1:500, 1:750 and 1:1000; the sixth mixture was reserved for control tests. These mixtures were tested at the end of 3, 9 and 20 days, using 5 cc. of the clear solution and 25 cc. of fresh one per cent starch paste. The results are given in Table XX.

TABLE XX.

		Mixtures of Malt Extract and Formaldehyde tested with Starch and Iodine at the end of		
Tube No.	Formaldehyde.	3 days.	9 days.	20 days.
1.	1:100	Bluish violet at the end of 2 hours.	Bluish violet at the end of 3 hours.	
2.	1:250	Light violet at the end of 2 hours.	Colorless at the end of 3 hours.	All the same as when tested on the 9th day.
3.	1:500	Nearly colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
4.	1:750	Colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
5.	1:1000	Colorless at the end of 2 hours.	Colorless at the end of 2 hours.	
C.	.....	Deep blue at the end of 2 hours.	Deep blue at the end of 2 hours.	

Experiment XXX.—An extract of malt diastase was prepared as described in Experiment XXVII. Formaldehyde was added to portions

of this in the proportions of 1:100, 1:250, 1:500 and 1:1000; another portion was reserved for control tests. The mixtures were allowed to stand at ordinary temperature for 24 hours. 5 cc. of each were then added to 25 cc. of a freshly prepared one per cent starch paste made from potato starch and the tubes were kept at 60°. When tested with iodine solution at the end of fifteen minutes, all showed complete conversion. These results did not seem to agree with those previously obtained. It was found, however, that formaldehyde in rather strong concentration will destroy malt diastase when allowed to act on the latter for a few hours at 60°. This, taken in conjunction with the fact that corn starch is not so easily digested as potato starch, will explain the apparent differences in the results.

On the following day the above tests were repeated, and at the same time another series was made with portions of the same mixtures of extract and formaldehyde which had been heated to 60° for four hours. The results are given in Tables XXI and XXII.

TABLE XXI.

Mixtures of Malt Extract and Formaldehyde Two Days at Ordinary Temperature), Tested with Starch and Iodine.

Tube No.	Formaldehyde.	Results.
1.	1:100	Colorless in about 20 minutes.
2.	1:250	Colorless in less than 15 minutes.
3.	1:500	" " "
4.	1:1000	" " "
C.	.....	Deep red at the end of 2 hours.

TABLE XXII.

Mixtures of Malt Extract and Formaldehyde (Kept at 60° for Four Hours), Tested with Starch and Iodine.

Tube No.	Formaldehyde.	Results.
1.	1:100	Deep violet at the end of 5 hours.
2.	1:250	Light violet at the end of 5 hours.
3.	1:500	Nearly colorless at the end of 2 hours.
4.	1:1000	Colorless in about 15 minutes.
C.	.....	Violet at the end of 5 hours.

It would seem from these results that, as already pointed out by Weigle and Merkel, the action of malt diastase is aided by the presence of formaldehyde. This, however, is due to the inhibition of bacterial growth by the formaldehyde. A glance at the tables shows that a solution of the ferment in water soon undergoes decomposition; after such a solution has stood for two or three days it is able to convert little if any of the starch. If formaldehyde is present in the proportion of

1:500 or 1:1000, the ferment does not decompose; on the other hand, it does not seem to be affected by the formaldehyde, for it is as active at the end of three weeks as when fresh.

CONCLUSIONS.

The following general conclusions may be drawn from the preceding work:

Fibrin is altered by formaldehyde and is then less easily digested by pepsin and by trypsin. Papaïn is apparently unable to digest fibrin even when this is exposed to very weak formaldehyde (1:1000) for a very short time.

The casein of milk, on contact with formaldehyde, undergoes rapid alteration and is as a result not coagulated by rennet, or but very slowly. Such altered casein, like similar fibrin, is not readily digested by the proteolytic ferments. The longer the formaldehyde acts on casein and on fibrin the more marked is the result.

Pepsin is not affected by a one per cent solution of formaldehyde, even when the mixture has stood for four weeks. Even a five per cent solution of formaldehyde acting for three weeks has no effect on pepsin. Contrary results obtained by others are due to an alteration of the fibrin by the formaldehyde. A putrid solution of pepsin in distilled water one month old digests fibrin as readily as a fresh solution.

Rennet is not affected even by a four per cent solution of formaldehyde acting for several weeks. The absence of coagulation at times is due to the action of formaldehyde on the casein of the milk and not on the rennet ferment.

Papaïn is very quickly altered by formaldehyde, even in very dilute solution. Moreover, it is unable to digest fibrin that has been exposed to the action of a very dilute solution of formaldehyde for a short time.

Trypsin is altered by formaldehyde to such an extent that digestion of fibrin will not take place, or but very slowly. The extent to which trypsin is affected by formaldehyde depends largely upon the amount of organic matter present, as well as on the amount of ferment in the solution.

Amylopsin is not destroyed by very dilute solutions of formaldehyde, but stronger solutions decrease the activity of the ferment, and if used in sufficient concentration will destroy it completely.

Ptyalin, like the diastatic ferment of the pancreas, is not destroyed by dilute solutions of formaldehyde. If the latter is used in rather strong concentration and allowed to act for some time it will destroy the ferment. The action of formaldehyde is more rapid and more marked at a slightly elevated temperature than at ordinary room temperature.

Malt diastase, unlike the diastatic ferments of the saliva and pancreatic solution, is not destroyed by formaldehyde when this is used in moderate amount and at ordinary temperature. Unlike pepsin, a solution of malt diastase readily undergoes decomposition on standing even for one or more days. This destruction is undoubtedly due to bacteria since it does not take place when formaldehyde is present. Consequently the favoring action which formaldehyde apparently exerts on diastase really consists in the inhibition of the growth of micro-organisms, and hence the diastase is protected against decomposition.

A SECOND CASE OF GONORRHŒAL SEPTICÆMIA AND ULCERATIVE ENDOCARDITIS WITH OBSERVATIONS UPON THE CARDIAC COMPLICATIONS OF GONORRHŒA.*

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PLATE I.

Cardiac complications of gonorrhœa with or without coincident or preceding arthritis, while not of frequent occurrence, are by no means so rare as has, even within recent years, been supposed. The literature shows over a hundred cases in which a diagnosis of gonorrhœal endo-, peri- or myo-carditis has been made, and during the last several years considerable attention has been attracted to the subject by the accumulation of evidence demonstrating the fact that many of these complications are due to actual local infections with the gonococcus.

It was the good fortune of Blumer and one of the writers to observe a case of gonorrhœal ulcerative endocarditis in 1895 and to succeed for the first time in obtaining the gonococcus in pure culture during life from the circulating blood; moreover organisms showing all the characteristics of gonococci were demonstrated in the lesions upon the affected valves, thus furnishing definite proof of the possibility of the existence of a true gonorrhœal septicæmia and endocarditis.

This case, which has already been reported,† it may be well to summarize:

The patient, a woman 34 years of age, entered the Johns Hopkins Hospital, April 25, 1895. Her family and personal history were nega-

* This case was reported at the Twelfth International Medical Congress at Moscow in August, 1897.

† *Arch. de méd. expér. et d'anat. pathol.*, 1895, vii, 701; also, *The Johns Hopkins Hospital Bulletin*, 1896, vii, 57.

tive, excepting that for three months she had had rheumatism off and on in various of her joints. From the beginning of her rheumatism she had complained of weakness and dyspnœa on exertion. A few days before her entry into the hospital she had a severe chill and took to bed. On entrance there were well-marked signs of mitral stenosis. During the period of her sojourn in the hospital there was irregular fever associated with severe chills. The blood showed throughout a well-marked leucocytosis; the urine contained a trace of albumin and the sediment contained occasional casts. The patient grew rapidly feeble and died May 16.

The diagnosis of ulcerative endocarditis having been made during life, cultures were taken from the blood on several occasions. These cultures were made by Dr. Blumer according to the method of Sittman. The blood, taken from the median basilic vein by a sterilized syringe, was mixed with melted agar which was immediately plated. Large quantities of blood were used, so that the medium contained at least one-third blood. The first culture, taken May 4, was negative, but in the cultures of May 7 and 12 the plates showed very minute white colonies representing a pure culture of small biscuit-shaped diplococci which failed to grow on transmission to agar-agar, gelatine, potato, bouillon or litmus-milk. These organisms were decolorized entirely by Gram's method.

The autopsy confirmed the diagnosis made during life, revealing an extensive ulcerative and vegetative endocarditis of the mitral valve. In the thrombi upon the valve were found large numbers of diplococci having all the morphological and tinctorial characteristics of gonococci. At the time of autopsy there were unfortunately no media at hand suitable for the cultivation of gonococci, and implantations, made upon agar-agar and ox's blood serum, from the heart's blood, valves, liver, spleen, lungs and kidneys, were entirely without result; it should be stated that in these post-mortem cultures but a small quantity of the heart's blood was mixed with the agar. Inoculation of a mouse with a piece of thrombus from the valves was without result.

The characteristic appearance and disposition of the cocci, their decolorization according to the method of Gram, their failure to develop upon ordinary media, and finally their growth on two occasions during life upon a medium practically the same as that recommended by Wertheim, leaves, it seems to us, little doubt that this was a true gonococcal infection. Similar organisms were found after death in the vagina and uterus.

Certain reviewers have been inclined to doubt the complete reliability of this observation. Thus, Fraenkel* asserts that "because during life a gonorrhœal affection was not discovered in the patient despite careful search, and cultures of the observed microörganism were not made on human blood serum or Wertheim's serum agar, the observation cannot be considered as entirely free from criticism." We confess that we cannot see the justice of Fraenkel's observations. It is well known to gynæcologists that gonorrhœal affections often exist in women without being recognized by the ordinary methods of examination. As was stated in the previous communication, we had not thought during the life of the patient of the possibility of the case being one of gonorrhœal infection, and the vaginal secretion was not examined. But after death characteristic gonococci, answering to all tinctorial and morphological characteristics, were found both in the vagina and the uterus. Moreover, the medium upon which the successful cultures were twice obtained during life—the mixture of blood fresh from the veins with melted agar—was essentially similar to the human blood-serum agar of Wertheim. Upon this medium the organism grew; upon all ordinary media it failed to reappear.

Shortly after the publication of the foregoing case we observed a second instance of endocarditis and septicæmia of undoubtedly gonorrhœal nature.

J. K., aged 19, a day laborer, unmarried, a native of Germany, was admitted to the Johns Hopkins Hospital on February 5, 1896, complaining of fever and weakness.

Family history.—Father died with dropsy; mother, one brother and two sisters living and well; several brothers and sisters died in infancy.

Personal history.—There is no history of the ordinary diseases of childhood. He has never had any severe infectious diseases; is sure that he has never had rheumatism or scarlet fever, stating that he has always been a healthy man. He drinks beer in moderation.

Present illness.—The patient contracted gonorrhœa for the first time six months ago. Several weeks after the onset he began to suffer from chilly sensations, fever and general weakness. Toward the end of November he began to have violent chills, occurring usually in the morning hours; these were followed by fever and profuse sweating.

* *Hygienische Rundschau*, 1896, vi, 254.

Under treatment the shaking chills disappeared, but the fever continued, becoming, however, more irregular. He has grown progressively weak and pale, and for two weeks before entry there has been œdema of the feet and ankles.

Physical examination.—The patient is very dull and drowsy. He is a large well-nourished man; lips, mucous membranes and skin extremely pale; pulse large, but of low tension, 108; respiration 30; T°, 103.4°. Lungs, clear throughout. *Heart:* Point of maximum impulse in the 4th space just inside the mamillary line. Relative dulness begins in the 3d interspace and is not increased to the right. Absolute cardiac dulness is obliterated by pulmonary resonance. On auscultation the first sound at the apex is booming and prolonged; there is no actual murmur. Passing toward the base a soft systolic murmur becomes audible; most marked in the pulmonic area. The second pulmonic sound is a little sharper than the second aortic. *Liver:* hepatic flatness begins at the 7th rib in the mamillary line, the lower border being palpable, 6.75 cm. below the costal margin. *Spleen* is greatly enlarged, flatness above beginning at the 7th rib, while the lower border is palpable 9.5 cm. below the costal margin. *Abdomen:* full, bulging a little in the flanks, tympanitic in the elevated, flat in the dependent parts; well-marked movable flatness. The left knee-joint contains an excess of fluid, being distinctly swollen and fluctuating. No redness or tenderness. No tenderness or irregularities on any of the long bones. Moderate enlargement of the inguinal glands. Slight œdema of the feet and ankles. There is a thick, purulent urethral discharge showing characteristic gonococci. The *blood* contains no malarial parasites or pigment. There is a moderate poikilocytosis. Red blood corpuscles, 2,292,000; colorless corpuscles, 9,000.

Urine: reddish amber; acid; 1015; no sugar; albumin, 0.1 per cent. Sediment: considerable; whitish; microscopically, numerous pus cells, usually separate, not in clumps; red blood corpuscles; small round cells about the size of leucocytes with single nuclei; numerous hyaline and granular casts with adherent pus and degenerated epithelial cells; epithelial casts; pus casts.

The patient remained in the hospital but nine days, during which time the temperature ranged between 99.6° and 103.3°. The urine was somewhat reduced in quantity, averaging a little under 1000 cc. in the 24 hours. The specific gravity ranged between 1013 and 1015, while the amount of albumin and the character of the sediment continued about as noted above.

11/ii/96. *Examination of the blood:* Red blood corpuscles, 2,283,000; colorless corpuscles, 14,250; hæmoglobin, 45 per cent. Dried specimens prepared according to Ehrlich's method showed slight variations in the size of the corpuscles, moderate poikilocytosis; a few nucleated red corpuscles, no malarial parasites. Differential count of leucocytes: small mononuclear leucocytes, 4.8 per cent; large mononuclear and transitional leucocytes, 2.6 per cent; polymorphonuclear neutrophilic leucocytes, 92.6 per cent; eosinophilic leucocytes, none.

The direct sequence of the symptoms upon the gonorrhœa suggested to us the possibility that we might be dealing with a gonorrhœal pyelo-nephritis and possibly with a general septicæmia, and cultures were taken on two occasions by Dr. Lazear from the circulating blood by the same method adopted in the previous case. These cultures were without result in both instances.

The patient was kept in bed, placed upon a milk diet, diuretics and iron. On February 14 he left the hospital, objecting to the strict régime.

March 9 the patient re-entered the hospital, having grown steadily worse.

10/iii/96. *Physical examination.*—The patient was extremely sallow, pale; tongue dry and fissured; pulse 108; moderate œdema of the dependent parts; slight puffiness of the face. The point of maximum cardiac impulse had moved outward and downward to a point in the 5th space slightly outside of the mamillary line, while a soft systolic murmur, which was not present on the former admission, was now to be heard all over the cardiac area, loudest at the apex. The second sounds at the base of the heart were not loud, but were of normal relative intensity; no accentuation of the second pulmonic sound.

The urethral discharge had almost disappeared.

The anæmia had increased, the *blood count* showing on 10/iii/'96: red blood corpuscles, 1,920,000; colorless corpuscles, 8500; hæmoglobin, 18 per cent.

The *urine* of the same date was of a pale but distinctly smoky color; acid; sugar absent; albumin 0.4 per cent. Sediment, abundant; microscopically, many pus cells scattered throughout the field, not arranged in clumps; many small round cells about the size of leucocytes with single nuclei; many red blood corpuscles, some "shadows," others crenated, others fairly well preserved. Numerous fatty degenerated epithelial cells, somewhat larger than pus cells; some small agglomerations of free yellow fat drops; occasional compound granular cells; casts

extremely abundant; hyaline, finely and coarsely granular with adherent pus and red blood cells; occasional fatty casts and blood casts; many epithelial and pus casts; occasional extremely large, slightly yellowish, typically refractive, waxy casts with broken ends; diazo-reaction absent.

The patient grew rapidly worse; the anæmia increased, the *blood* on the day of death showing: red blood corpuscles, 1,896,000; colorless corpuscles, 18,000; hæmoglobin, 18 per cent.

The *urine*, averaging little over 600 cc. for the 24 hours, was almost suppressed during several days before death. The albumin increased up to nearly 0.5 per cent, while the number of fatty, blood, waxy, epithelial and pus casts increased. On March 23 the paient became extremely dull and drowsy.

23/iii/96. (Professor Osler.) "For the past few days the temperature has been lower, not above 100° since the 19th; no change in the general condition. Pulse about 100; drops a beat occasionally; of low tension.

Heart: apex beat is diffuse during expiration; well seen in the 5th space and a little outside the nipple line. Cardiac impulse is visible in the 4th space inside the nipple. A diastolic shock can be felt at the apex; both sounds are audible; no murmur. Over the entire praecordium there is a to-and-fro superficial pericardial friction murmur, the maximum of which is at the 5th left cartilage; it is well heard at the ensiform cartilage; not heard above the level of the 3d rib; no especial accentuation of the pulmonic second sound."

On the 24th the patient developed a well-marked petechial eruption.

25/iii/96. (Dr. Thayer.) "The patient is lying on his right side; very drowsy and dull. Respirations 15 to the minute, rather deep and noisy; pulse 21 to the quarter; of low tension. Face puffy; pupils not contracted; general anasarca.

Heart: diffuse heaving over the 4th and 5th interspaces just inside the nipple; the point of maximum cardiac impulse is not to be sharply differentiated; flatness does not pass the left sternal margin; begins at about the 4th space. At the apex the first sound is reduplicated and followed by a soft systolic murmur, while in connection with this there is a soft superficial to-and-fro rub. Over the body of the heart the sounds are considerably masked by this friction rub. *The second pulmonic sound is, however, not accentuated.* On the trunk and arms and occasionally on the legs are numerous small petechial spots, the largest scarcely larger than the head of a pin."

On March 21 the patient began to suffer from diarrhœa, the movements

becoming gradually more frequent and fluid. During the afternoon of the 25th the respiration became more stertorous; the patient lapsed into a condition of complete coma and died at 6.30 P. M.

Bacteriological examination during life.—March 22, 1896, cultures were made by Dr. Lazear in the following manner: 2 cc. of blood were drawn from the median basilic vein by a hypodermic syringe previously sterilized by boiling. The skin was, as far as possible, sterilized and every antiseptic precaution was used. The blood was mixed with 4 cc. of melted nutrient agar and the mixture poured into a Petri dish and allowed to harden. It was kept in a thermostat at 35° C. At the end of 24 hours no growth was visible. At the end of 48 hours there began to appear colonies half the size of a pin head, granular in appearance with somewhat irregular borders. These colonies were made up of cocci arranged usually in pairs. The cocci were of biscuit or kidney shape, their flattened sides being adjacent. They stained well with the ordinary basic dyes and decolorized by Gram's method. Transplanted upon human blood-serum agar by a smear upon the surface there developed a fair number of colonies similar to the above, consisting of diplococci having the same morphology and tinctorial characteristics. Transplanted to ordinary agar there was a growth of a few fine colonies of the same diplococci. On gelatine, ox's blood serum and bouillon there was no growth. At the end of ten days the organisms had all died out upon the original plates.

On March 24 and 25, plate cultures were made by the same method, and on each occasion there was an abundant growth, in pure culture, of diplococci identical with those obtained upon the first plates, behaving in the same manner with regard to stains and in their growth upon the various culture media.

On the basis of these positive cultural experiments and the clinical history of the case, the patient was brought before the class on March 25 as an instance of general gonococcal septicæmia with endo- and pericarditis.

Autopsy, March 25, by Professor Flexner.

Anatomical diagnosis.—Gonococcal septicæmia; subacute gonorrhœa; subacute ulcerative and vegetative, tricuspid endocarditis caused by the gonococcus; subacute splenic tumor; chronic passive congestion of the liver; subacute hæmorrhagic and glomerular nephritis; acute sero-purulent pleurisy and pericarditis due to the gonococcus; pulmonary infarct.

The following is a summary of the pathological record:

On opening the abdomen nothing remarkable was to be made out

except the much enlarged spleen, adherent in its upper part to the diaphragm.

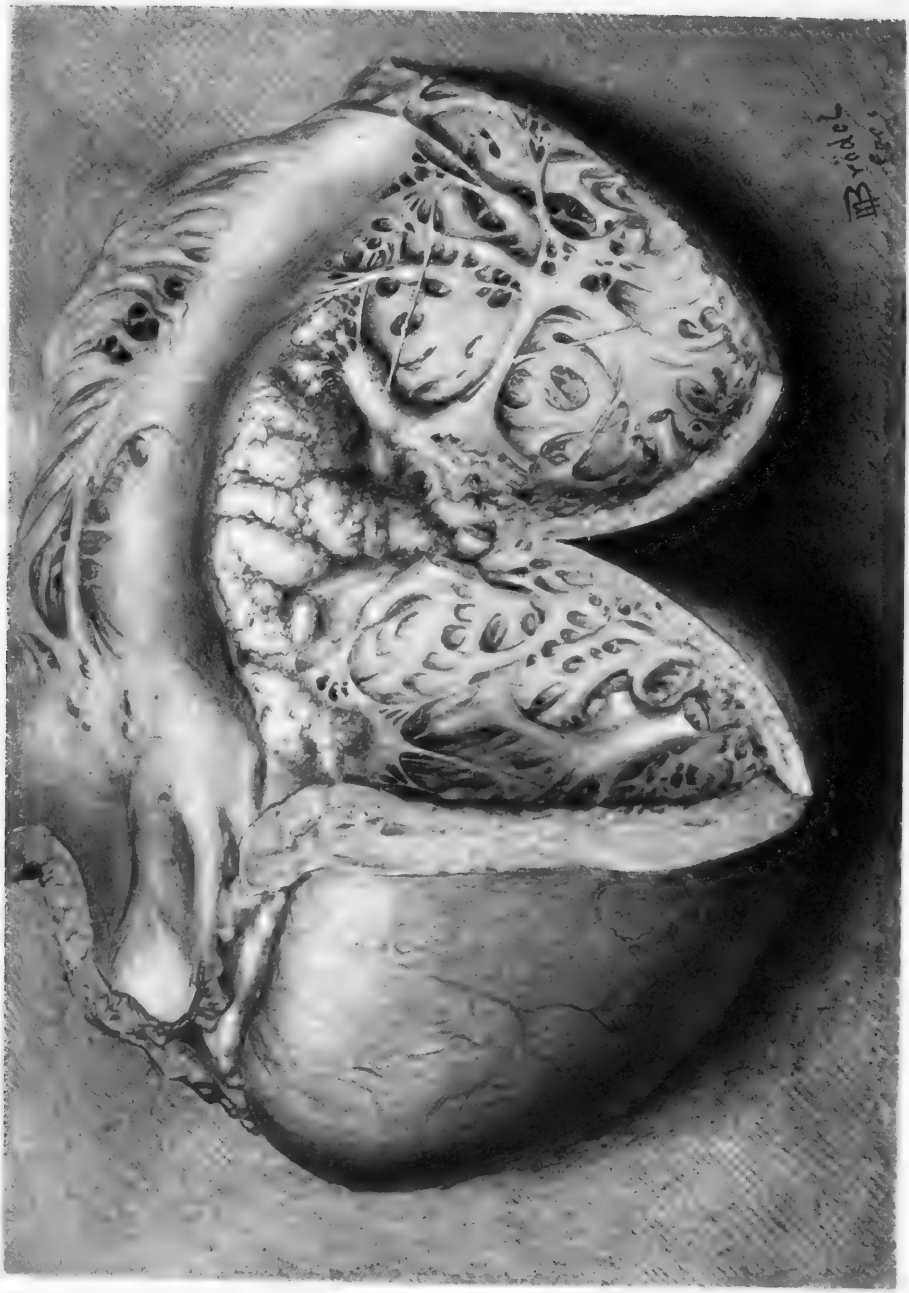
Pleurae and lungs.—The right pleural cavity is free from adhesions and contains about 800 cc. of slightly turbid, yellowish fluid with large flakes of fibrin. On the pulmonary pleura there are small flakes of fibrin and beneath this are many punctiform ecchymoses. The left lung is bound down along its posterior border by firm adhesions. In the left pleural cavity are 550 cc. of fluid containing somewhat less fibrin than on the other side. There are less fibrin and fewer ecchymoses on the visceral pleura than on the right side.

In the middle of the left lower lobe at its inferior border there is a circumscribed, triangular area of infarction 1½x1½ cm. in extent; opaque, firm, of a brownish-yellow color. The surrounding tissue is congested. On section both lungs are of a light salmon-red color and œdematous. The bronchi are congested. The pulmonary arteries are free and practically normal in appearance. The anterior edges of the lungs are emphysematous.

Pericardium and heart.—The pericardial cavity contains about 300 cc. of turbid yellow fluid with fibrinous flakes. In the dependent portions the fluid is thick and puriform. The surface of the peri- and epicardium is congested, ecchymosed, and covered by a granular deposit. The weight of the heart and pericardium is 680 grammes.

The right ventricle and auricle are dilated and contain fluid blood and partly decolorized clots. The endocardium of the right auricle is delicate, although near the obliterated foramen ovale beneath the endocardium are two or three slightly elevated minute opaque points.

The tricuspid valve is the seat of an extensive thrombus, occupying the entire middle segment, with the exception of its base, and to a less extent the two remaining segments (see Plate I). The thrombus attached to the middle segment is firmly united to the valve at a distance of 5 mm. from its attachment to the auriculo-ventricular ring and projects into the cavity of the ventricle. At the site of attachment of the thrombus the substance of the valve is destroyed. The thrombus presents an irregularly convoluted appearance, and for description may be divided into three distinct portions: The central portion, which is largest, measuring 4.5x2 cm. x4.5 mm. in thickness, has in general a conical shape with its base at the valve and its apex projecting into the ventricle. To the left of this is a second mass 18x22 mm. in extent, almost quadrangular in form but irregular in contour. The remaining mass to the right is about one-half the size of the last. This thrombus



mass is attached at certain points to the endocardium of the right ventricle. Fully one-half of the chordæ tendineæ of the segment of the valve are ruptured and their free ends are covered with grape-like clusters of thrombi. Small miliary vegetations are on the papillary muscle to which these are attached and on a moderator band which extends from the papillary muscle to the mid-portion of the left segment of the valve. The anterior surface of the right segment of the valve is the seat of two thrombus masses projecting into the auricle; they average about 12x10 and 12x6 mm. The pulmonary artery and valves are normal in appearance.

The aortic and mitral valves and the endocardium of the left ventricle are entirely normal. In the lymphatics along the course of the vessels in the epicardium on the right side there are minute discoid nodules similar to those described in the endocardium of the auricle.

The tricuspid orifice measures 16 cm.; length of the right ventricle 12.5 cm.; thickness 5-7 mm. The mitral valve measures 12 cm.; length of the left ventricle 8 cm.; thickness 15 mm. The cardiac muscle is pale and fairly firm.

The *spleen* weighs 840 grammes; dimensions, 21x13x6 cm.; capsule wrinkled; on section the Malpighian bodies very prominent, the trabeculæ visible; the pulp of an opaque grayish-red color, the consistence moderately firm.

The *liver* weighs 2450 grammes, and is the seat of fairly well-marked chronic passive congestion.

Kidneys.—The kidneys weigh together 670 grammes. Dimensions: right, 14x9.5x4; left 15x8x4. They are swollen, the capsule adherent in places; the surface mottled, of an opaque, grayish color with many punctiform hæmorrhages. The cut surface appears swollen and œdematous. The striæ are obscure; the glomeruli visible, but pale. Numerous elongated hemorrhages—9 to 10 mm. long—are visible in the cortex. The pyramids are hyperæmic; the vessels of the mucous membrane of the pelvis are also somewhat congested and a few small hæmorrhages are visible.

The *ureters* are not enlarged; their mucous membrane shows the same condition as that of the renal pelvis.

Bladder.—The vesical mucous membrane is pale, with the exception of the trigonum, where it is moderately congested. About the orifices of the ureters there are a few small hæmorrhages. The neck of the bladder and the prostatic portion of the urethra are somewhat hyperæmic.

Gastro-intestinal tract.—The stomach and intestines show nothing noteworthy.

Pancreas.—A number of small opaque areas of fat necrosis are to be made out in the fatty capsule of the pancreas. Teased preparations show in the necrotic areas groups of fat cells containing finely granular bodies. Treated under the microscope with concentrated sulphuric acid, gas bubbles may be seen to arise and soon fine crystals of calcium sulphate appear.

Bacteriological examination.—Cover-slips from the pleural and pericardial exudates and from the vegetations upon the heart valves showed large numbers of cocci in pairs. They were excessively numerous upon the surface of the tricuspid valve. These cocci were in almost all instances included within polymorphonuclear leucocytes which were numerous. The form of the cocci was, as a rule, typically biscuit-shaped, and at times, though rarely, two pairs lay side by side, suggesting a tetrad arrangement. The cocci stain readily in the usual aniline dyes, but are quickly and uniformly decolorized by Gram's method.

A cover-glass specimen from the spleen showed one pair of cocci; others were not found. Cover-slips from the kidney, renal pelvis and urinary bladder were negative.

In the urethra, among a variety of bacilli, definite intracellular biscuit-shaped diplococci which decolorize by Gram were found.

Cultures.—Cultures were made at the autopsy from the various local exudates, the heart's blood and the organs as follows:

(*a*) Upon Loeffler's blood serum, prepared; (1) from human blood; (2) from bullock's blood; (3) from dog's blood.

(*b*) Upon agar-agar. Only a few tubes of the human serum were on hand and these were used for the local exudates and blood. Upon those from the pericardium, tricuspid valve and heart's blood delicate growths were obtained which consisted in part of confluent minute colonies, in part of small, almost point-like, grayish-white, slightly elevated colonies. Microscopically these colonies were composed of diplococci, readily decolorizing by Gram, and resembling in every way, except perhaps in the feebleness of their growth, the organisms isolated during life. No growths whatever were obtained upon bullock's serum, dog's serum, or plain agar-agar.

Transplantations from the 48-hour old growths from the human blood serum on to ordinary agar, swine-liver agar (Livingood), fœtus agar (Flexner) were negative. As no human serum remained, transplantations entirely failed.

Microscopical examination.—A histological study of the organs was made by Professor Flexner, to whom we are indebted for the following report: The description of the histological changes is confined to those of the kidneys, the liver, the spleen and the cardiac valves, these being the parts chiefly affected. The tissues were hardened in alcohol and Zenker's fluid, and the specimens stained by hæmatoxylin and eosin, Weigert's fibrin stain, and methylene-blue.

Kidney.—Throughout the cortex there is a general increase of connective tissue, uniformly distributed and particularly well marked between the tubules. This tissue is fibrillated, œdematous and not particularly rich in cells. The chief lesions affect the glomeruli and the labyrinthine tubules. The least affected glomeruli completely fill the capsule of Bowman; the number of cells within the glomerular capillaries is increased, this increase being due to an excess of polymorphonuclear leucocytes. The capillary walls are distinct, thicker than normal, hyaline or slightly fibrillated in appearance. The more abnormal glomeruli show, in the first place, thrombosis of groups of capillaries by material presenting characters unmistakably indicative of fibrin. This material within the capillaries appears as a delicate network and may be limited to a single loop of a capillary or occupy a group of loops; it does not completely occlude the vessels. Outside of the capillary walls there is an increase in the number of cells within the glomerular space. These cells are partly of an epithelioid type, being doubtless derived from the capsular and glomerular epithelium, and partly leucocytes. Deposited within and intimately mixed with these cells a fibrinous material, which appears often as a crescentic band, dips down between the lobules of the glomeruli. It is in part distinctly fibrillated, in part dense and hyaline, and everywhere gives a sharp staining reaction for fibrin. Its association with the cells within the capsular space is of the most intimate character. In specimens stained with hæmatoxylin and eosin it takes a vivid red stain. In not a few situations there is evidence of a proliferation of cells clearly derived from the capsular epithelium, and, although less certainly marked, there is in our mind little doubt that a similar increase of cells is taking place within the capillaries themselves.

As has been stated, leucocytes occur abundantly in the capsular spaces; these are derived from the glomerular capillaries and may be seen in the act of migration through the capillaries into the space. Of particular interest is the passage of leucocytes from the capsular space through the capsule of Bowman into the interstitial tissue in which these

cells are increased. The uriniferous tubules contain also large numbers of polymorphonuclear leucocytes; in some places completely occluding the lumen of the tubes, forming definite leucocytic casts. The epithelium, especially of the secreting tubules, is much degenerated, even necrotic, and in some places evidently proliferating, as is evidenced by multinucleated cells in certain of the tubules. The degeneration of the epithelium is partly fatty and granular, but largely of the hyaline variety, to which change can be attributed much cast material and definite casts occupying the tubules. Red blood corpuscles are rarely found within the tubules. The essential lesion is a sub-acute glomerular and intracapillary nephritis. Bacteria were not discovered in this organ.

Spleen.—The connective tissue framework of the spleen is thickened. The new tissue is of a semi-fibrillated character. The blood-vessels of the pulp are diminished in size and apparently also in number. The splenic elements proper are also reduced in number, but there are scattered irregularly throughout the spleen in greatly increased number polymorphonuclear leucocytes, and within the venous sinuses groups of hyaline bodies and single hyaline bodies, globular in shape, varying in size from a red blood corpuscle to one of the largest white cells there present.

The follicles are enlarged and very distinct, many of their cells being swollen and in process of division; they are infiltrated with polymorphonuclear leucocytes, the nuclei of many of which show the greatest irregularity in form. Nuclear fragments are scattered sparsely and irregularly throughout the spleen; for the most part they are not enclosed within other cells.

Liver.—The connective tissue is not increased; the central veins are much dilated and the central portions of the lobules hyperæmic. This congestion varies in different lobules, being in some very marked, with corresponding atrophy of the liver cells. There is an interesting hyaline metamorphosis of some of the liver cells. Those peripherally placed in the congested areas show this change best, and in these the nuclei have become small, contracted and deeply staining. In parts of the liver distant from the congested areas the hepatic cells are swollen, fatty, and free from the hyaline change described, excepting where an occasional cell, more or less loosened from the rows of liver cells and perhaps lying in the capillary, is thus affected. There is a general increase in the number of leucocytes within the blood-vessels.

Heart.—Sections through the tricuspid valve include chiefly the thrombus, which consists of masses of blood platelets, of fibrin and of

included leucocytes. No bacteria appear in sections stained by Gram's or Weigert's method.

The pericardium shows, besides a superficial fibrinous exudate containing leucocytes, a considerable inflammatory infiltration of the subjacent fibrous tissue and a proliferation of the fixed tissue cells. The epithelial covering is in active proliferation. In some of the leucocytes in the pericardial exudate Dr. Lazear was able to demonstrate gonococci.

A further study of the heart valves was made by Dr. Lazear: The tissues were hardened in alcohol and in Zenker's fluid, and embedded in celloidin and paraffin. For the bacteriological study they were stained with Loeffler's and Unna's methylene-blue, Ziehl-Neelsen carbolic fuchsin, Stirling's gentian violet and by Gram's method. For histological study they were stained with hæmatoxylin and eosin and Weigert's fibrin stain.

Tricuspid valve.—The connective-tissue structure of the valve shows areas of necrosis. In places the nuclei are merely swollen. There are also irregular areas extending throughout the valve in which the nuclei have entirely disappeared, leaving a homogeneous material which stains with eosin. Extending into the substance of the valve are numerous spaces filled with leucocytes, among which are a few large phagocytic cells, with large irregularly shaped nuclei and containing red blood corpuscles.

The vegetations are made up of granular masses of platelets surrounded by fibrin and a thick layer of leucocytes. Leucocytes are present also within the masses of platelets, either scattered or occupying spaces.

Upon the surface of the valve and the vegetations is a layer composed of leucocytes and red blood corpuscles with strands of fibrin forming an irregular network through it. The same material occupies the interstices between the vegetations. Many of the leucocytes in this layer and in the more central portions contain micrococci with the typical biscuit shape and other morphological characters of gonococci and completely decolorizing by Gram. Extracellular gonococci are present in the central portion of the thrombi but not in the superficial layers.

At the line of junction of the central platelet mass and the leucocytic layer is a long line of globular bodies varying in size from that of a leucocyte to ten times this size. These bodies are made up of dense masses of gonococci.

There can, it appears to us, be no doubt as to the nature of the organisms obtained in pure culture from the blood during life and

from the affected valves, heart's blood and pericardium after death. This case and that previously reported by Dr. Blumer and one of the authors (Thayer) are the first two recorded instances in which absolute proof of the gonococcal nature of the general infection has been obtained.

The case presents several interesting and unique features. While the diagnosis of ulcerative endocarditis and gonorrhœal septicæmia was made during life, the exact anatomical lesion—the remarkably extensive affection of the tricuspid valve—was not at this time suspected. Much of the thrombus upon the valve may have been of relatively recent formation, but older changes, probably of several months' duration, were clearly present. It is the first instance in which a pure tricuspid lesion has been found in a gonorrhœal endocarditis. It is not uninteresting that while the diagnosis was not made clinically, it was particularly noted that, in association with the systolic murmur, there was no accentuation of the second pulmonic sound, a fact which might well have excited our suspicion.

The changes in the kidney are of especial interest. Clinically the case presented the features of a grave acute nephritis with anæmia, anasarca, ascites and finally uræmic coma. The urine showed, besides the large quantity of albumin, the blood casts and the epithelial cells, so large an amount of pus that a diagnosis of probable pyelitis or pyelo-nephritis was made. Especially interesting is the fact that during life casts consisting entirely of polymorphonuclear leucocytes were repeatedly observed. The absence of gross collections of pus in the kidney or in the pelvis was a surprise at the time of the autopsy. The microscopical examination of the kidney, however, revealed the source of this pus in the unquestioned evidence of the passage of numerous leucocytes directly through the capillaries of the glomeruli into the glomerular spaces and the urinary tubules. The extensive thrombosis of the glomerular capillaries and the accumulations of fibrin in the glomerular spaces form a very remarkable picture. How this special localization of these changes in the kidney and the relative freedom of the liver from those degenerative processes so common in general septicæmia, viz. focal necroses, may be explained is rather

an interesting question. May it perhaps be true that the renal changes were due to the direct deleterious action of the gonococci or their products in the process of elimination through the urine?

That the gonococcus must be recognized as a true pyogenic organism capable of giving rise to the gravest local and general septic complications has been abundantly proven by recent observations. That cystitis, epididymitis, spermatocystitis, prostatitis and periurethral abscesses in man, metritis, vulvo-vaginitis, salpingitis, and peritonitis in woman, may owe their origin solely to the presence of the gonococcus has been clearly demonstrated. And further, suppurative processes in remote parts are now known to be occasionally due to a pure gonorrhœal infection. Horwitz and Lang* found the gonococcus in an ulcer upon the back of the hand, while its presence in joint fluids in cases of gonorrhœal arthritis has been demonstrated by many observers. Within the past 15 months Dr. Young has obtained the gonococcus in pure culture in 7 instances of gonorrhœal arthritis in the Johns Hopkins Hospital, demonstrating that the arthritis in a large proportion of instances represents a true local bacterial infection.

The gonococcus has also been obtained in pure culture from a number of instances of tenosynovitis (Jacobi and Goldmann,† Bloodgood and Young‡), from subcutaneous abscesses (Lang and Paltauf§ and Horwitz‖), from intramuscular abscesses (Bujwid¶), from pleural effusions (Bordoni-Uffreduzzi**), from the circulating blood (Thayer and Blumer,†† Thayer and Lazear‡‡), and recently by Young§§ from a case of general peritonitis following an acute gonorrhœal

* *Wiener klin. Wochenschr.*, 1893, vi, 59.
† *Beiträge z. klin. Chir.*, 1894, xii, 827.
‡ Unpublished observations in the Johns Hopkins Hospital.
§ *Arch. f. Derm. u. Syph.*, 1893, xxv, 330.
‖ *Wien. klin. Woch.*, 1893, vi, 59.
¶ *Centrlb. f. Bakt.*, 1895, xviii, 435.
** *Deutsche med. Woch.*, 1894, xx, 484.
†† Op. cit.
‡‡ *Med. Record*, N. Y., 1897, lii, 497, and present article.
§§ Unpublished observation.

salpingitis. That in some instances, therefore, the gonococcus itself should be the cause of endocarditis, pericarditis, and myocarditis is by no means remarkable. Our own experience during the last several years, together with a study of the literature, indicates that the cardiac complications of gonorrhœa are more frequent than has generally been supposed.

Endocarditis is by far the commonest of the cardiac complications of gonorrhœa. Gurvich* has collected 110 instances in 77 of which the cases are sufficiently well reported to allow of definite conclusions. From the more recent literature it is possible to add some ten or a dozen more cases to those of Gurvich.

In the majority of these cases the cardiac complications have been preceded by an arthritis. In a considerable number of instances, however, joint symptoms have been entirely absent, while in several cases evidences of endo- or pericarditis have appeared before the development of joint manifestations.

Pericarditis is a much less frequent complication. We have been able to collect but 17 positive cases in the literature.

Myocardial changes have been demonstrated in the majority of the cases of acute ulcerative endocarditis of gonorrhœal origin which have come to autopsy, the most satisfactorily studied instance being that of Councilman, where there was no affection of the endocardium.

For a satisfactory study, however, of the cardiac complications of gonorrhœa one must turn to the cases which have been observed anatomically as well as clinically. We have collected 32 instances of gonorrhœa with fatal cardiac complications where there were satisfactory pathological notes. Several of those included in other classifications have been omitted because of insufficient data. Of these 32 cases 31 were instances of ulcerative endocarditis with or without marked pericardial or myocardial affection; one, that of Councilman, was an instance of peri- and myocarditis alone.

Gonorrhoeal endocarditis.—These cases considered from a pathological standpoint may be divided into five classes:

* *Russk. arch. patol., klin. med. i. bakt.*, 1897, iii, 329.

(1) The first class includes six cases, those of Bourdon,* Desnos,† Schedler,‡ Draper,§ Fleury,‖ and His.¶

In these instances, although the history clearly shows the association of the process with gonorrhœa, no note is made with regard to bacteriological examination.

(2) The second class comprises ten cases, those of Martin,** Weckerle,†† Weichselbaum,‡‡ Ely,§§ Wilms,‖‖ Golz,¶¶ Keller,*** Zawadzki and Bregman,††† Babes and Sion,‡‡‡ and lastly an unpublished observation of our own, Case 32 of our series (p. 115). Here there existed mixed or secondary infections. In some instances organisms other than gonococci were obtained in pure culture; in others, strepto- and staphylococci were demonstrated microscopically. In several cases organisms morphologically similar to gonococci were found, while the actual sequence of the infection upon acute gonorrhœa is not to be doubted.

(3) The third class includes four cases, those of Rothmund,§§§ His,‖‖‖ Winterberg¶¶¶ and Fressel.**** Here the infection was probably purely gonococcal. In all of these cases organisms showing the morphological and tinctorial characteristics of gonococci were

* *Gaz. d. Hôp.* Par., 1868, xli, 1.

† *L'Union méd.*, 3s., 1878, xxv, 43; also, *Gaz. d. Hôp.*, 1877, l, 1067.

‡ "Zur Casuistik der Herzaffectionen bei Tripper." Inaug.-Diss., Berlin, 1880.

§ *Medical Bulletin*, Phila., 1882, iv, 81.

‖ *Journ. de méd. de Bordeaux*, 1883-84, xiii, 65.

¶ *Berl. klin. Woch.*, 1892, xxix, 993.

** *Rev. méd. de la Suisse Romande*, 1882, ii, 308, 352.

†† *Münch. med. Woch.*, 1886, xxxiii, 563, 582, 608, 622, 636.

‡‡ *Centralbl. f. Bakt.*, 1887, ii, 209.

§§ *Med. Record*, xxxv, 1889, 287.

‖‖ *Münch. Med. Woch.*, 1893, xl, 745.

¶¶ Ulceröse Endocarditis der Klappen der Pulmonalarterie bei gonorrhoischer Arthritis, Inaug.-Diss., Berlin, 1893.

*** *Deutsch. Arch. f. klin. Med.*, 1896, lvii, 387.

††† *Wien. med. Woch.*, 1896, xlvi, 313, 351.

‡‡‡ *Arch. d. Sc. Méd. de Bucarest*, 1896, i, 505.

§§§ Endocarditis ulcerosa, Inaug.-Diss., Zürich, 1889.

‖‖‖ Op. cit.

¶¶¶ Festschr. z. 25 Jähr. Jub. d. Vereins Deutscher Aerzte zu San Francisco, 1894, 40.

**** Inaug.-Diss., Leipzig, 1894.

demonstrated microscopically. Cultures, however, were not made. Other organisms were not found.

(4) The fourth group comprises six cases, those of Leyden,* Finger, Ghon and Schlagenhaufer,† Hale White,‡ Michaelis,§ Stengel ‖ and Siegheim.¶ Here the proof of the purely gonococcal nature of the process is more nearly complete in that the observers made cultures upon ordinary media from the affected parts and from the circulating blood without obtaining positive results; while at the same time the microscopical demonstration from the affected regions of organisms showing all the morphological and tinctorial characteristics of gonococci would seem to form a fairly conclusive argument in favor of the existence of a pure infection by the gonococcus.

(5) Lastly, there remain five cases, those of Thayer and Blumer,** Dauber and Borst,†† Thayer and Lazear,‡‡ Rendu and Hallé§§ and Lenharz,|||| in which the evidence of the purely gonococcal nature of the complication may be considered as definitely proven.

In the first of these cases the gonococcus was obtained in pure culture twice during life and was found microscopically post-mortem in the affected regions.

In the case of Dauber and Borst a pure culture was obtained after death from the heart's blood of organisms concerning the nature of which the reporters were in doubt. Most subsequent observers have, however, recognized them as gonococci.

In the third instance, the writers were able to prove the purely gonorrhœal nature of the affection by obtaining gonococci in pure culture three times during life from the circulating blood, while Dr. Flexner obtained similar results post mortem from the affected valves, heart's blood, and pericardium.

* *Deutsch. med. Woch.*, 1893, xix, 909.
† *Arch. f. Dermat. u. Syph.*, 1895, xxxiii, 141, 323.
‡ *Lancet*, 1896, i, 533.
§ *Zeitschr. f. klin. Med.*, 1896, xxix, 556.
‖ *Univ. Med. Mag.*, Phila., 1897, ix, 426.
¶ *Zeitschr. f. klin. Med.*, 1898, xxxiv, 526.
** Op. cit.
†† *Deutsch. Arch. f. klin. Med.*, 1896, lvi, 231.
‡‡ *Med. Record*, N. Y., 1897, lii, 497 (case reported in this communication).
§§ *Bull. et mém. Soc. méd. d. hôp. de Par.*, 1897, 3. s., xiv, 1325.
|||| *Berl. klin. Woch.*, 1897, xxxiv, 1138.

In the case of Rendu and Hallé gonococci were obtained in pure culture from the endometrium during life and demonstrated microscopically in the phlegmon about the elbow. After death they were obtained in pure culture from the thrombus upon the affected valves.

Finally the last link in the chain of evidence has been supplied by Lenharz, who, from a case of characteristic gonorrhœal endocarditis, in which pure cultures were obtained from the thrombi on the aortic valves, introduced a piece of softened thrombus into the human urethra. A characteristic gonorrhœa, with gonococci in the discharge, appeared on the fourth day.

Anatomical lesions.—As to the nature of the anatomical lesion there is little to say. Considering the 15 cases in which the pure gonococcal nature of the infection is probable, one finds that in all instances there were present the usual appearances of ulcerative endocarditis with extensive polypoid chrombi and more or less actual destruction of the valves, often with aneurism formation and perforation. The localization of the affection, however, presents several points of considerable interest. In ordinary chronic endocarditis it is well known how infrequently the right heart alone is affected; thus, out of 300 autopsies on cases of endocarditis, Sperling found the lesions limited to the right heart in but 3 instances, or 1 per cent. Weckerle out of 846 autopsies on cases showing valvular cardiac lesions in Bollinger's laboratory found that the right heart alone was affected in 3.9 per cent of all cases.

It has, however, been shown that in cases of ulcerative endocarditis the liability of the right side to infection is considerably greater. Thus, while in 802 benign cases from the Munich statistics the right heart alone was affected in 3.24 per cent, in 44 cases of ulcerative endocarditis the percentage of affections limited to the right side was nearly 16 per cent (15.91 per cent). In our 31 cases the lesions were as follows:

Left heart:	aortic	12	
	mitral	6	
	both	3	
		21	67.7%
Right heart:	pulmonary	7	
	tricuspid	1	
		8	25.8%
Both sides:	mitral, aortic, tricuspid	1	
	all four valves	1	
		2	6.4%

This surprisingly high percentage of right-sided cardiac affections in our cases is interesting and difficult to explain. Considering the 15 cases in which the pure gonococcal nature of the infection is probable, we have the following table:

Left heart:	aortic	7	Right side:	tricuspid	1
	mitral	2		pulmonary	2
	both	2			3—20%
		11—73.3%			

Both sides: all four valves.....1-6.6%.

The remarkable high percentage of pure right-sided cardiac affections in these cases, even as compared with the Munich tables, is an interesting point. To attempt to draw definite conclusions from so small a number of instances might well be fallacious, but the fact is none the less worthy of reflection. Indeed, why the right side of the heart should be so much more liable to disease in ulcerative endocarditis is by no means perfectly clear. Another very interesting point is the fact that the aortic valves appear from our tables to be by far the most frequently affected. While this has been the case in those fatal instances which have come to autopsy, yet Gurvich's tables based upon 64 cases in which a diagnosis was made during life show appreciably different figures. His tables show:

Mitral valve	31	Pulmonary	2
Aortic	16	Mitral and tricuspid	2
Mitral and aortic	13		

Age.—The age of the patients in the fatal cases varied between 19 and 51, while taking the larger statistics of Gurvich we find records of one instance at the age of 64 and another at 10. The majority of cases occurred, as might be expected, in early adult life.

Sex.—Of the 31 instances of fatal ulcerative endocarditis due to the gonococcus 23 were in men and 8 in women.

Time of onset.—There appears to be nothing particularly characteristic with regard to the time of onset of these cases with relation to the attack of urethritis, some, as in the case of Prévost, coming on almost immediately after the onset, others some weeks or months later. Some of the cases occurred with the initial attack of gonorrhœa, others in patients who had suffered once or twice before.

Complicating arthritis.—Arthritis preceded the cardiac affection in the majority of instances, though in a considerable number the cardiac complication occurred without or before the development of joint symptoms.

Symptoms.—The symptoms of gonorrhœal endocarditis appear to differ in no essential way from those of endocarditis of other origin. While many instances are reported in which the cardiac manifestations were slight and transient, the proportion of cases of a malignant nature which have so far been recognized is unusually large. It is not improbable, however, that this is due to the fact that the majority of milder cases escape notice. And it is well in this connection to remember how frequently the same is true in rheumatic cardiac affections.

In the fatal cases the symptoms are essentially similar to those in instances depending upon infection with other pyogenic organisms —an irregular intermittent or remittent fever, usually with severe rigors, profuse sweating and rapidly developing anæmia, albuminuria; in fact, the ordinary symptoms of a severe septicæmia. The duration of the attack in the 15 instances in which the pure gonococcal nature of the infection is probable, varied, as far as can be made out, from ten days to two months. The only exception to this rule was in our case, in which the symptoms of septicæmia lasted through a period of six months. From the consideration of these cases and of those others which were followed by recovery, it is rather difficult to see upon what Gurvich bases his conclusion that gonorrhœal cardiac affections pursue a milder course than those depending upon the other pyogenic cocci.

In a number of these instances the renal changes have been particularly severe. In several the patient died with the general appearances of a grave nephritis as in our second case. These changes are possibly to be explained by the special exposure of the kidney through the elimination of the gonococcus or its products which, in these instances of general septicæmia, occurs in all probability through the urine.

Pericarditis.—Pericarditis is a far less frequent occurrence than

endocarditis in gonorrhœal infection. In 7 of the 32 fatal instances there is a note of a pericardial complication. The cases of Weckerle and Golz were mixed infections. In Councilman's case the pericardial cavity was enormously distended, containing 800 cc. of a hæmorrhagic exudate in which there were large masses of clot. Both surfaces of the pericardium were covered with thick membranous masses containing hæmorrhages. A few gonococci were found in the pericardium. In Winterberg's case it is noted that 20 ccm. of sero-purulent fluid were found in the pericardial sac. In our second case the pericardial cavity contained about 300 ccm. of turbid yellow fluid containing flakes of fibrin. In the dependent portions the fluid was thick and puriform. The surface of the peri- and epicardium was congested and contained small ecchymoses; it was covered by a granular deposit. Gonococci were obtained in pure cultures from the fibrin upon the surface of the pericardium. In Rendu's case it is noted that the pericardium contained 500 cc. of translucent fluid and that there were "pericardial lesions," nothing further. Cultures from the fluid were negative.

Thus, it may be seen that anatomically nothing striking is to be made out from a consideration of these observations. The definite proof of the existence of a pure gonorrhœal pericarditis is furnished by the positive result of our cultures in Case II.

Myocarditis.—Grave myocardial changes, necroses with hæmorrhage, leucocytic infiltration, embolic abscesses have been described in a number of instances in association with fatal endocarditis. In Councilman's case the areas of necrosis and suppuration were large and gonococci were found microscopically in the foci.

CONCLUSIONS.

(1) An acute gonorrhœal urethritis may be the starting point for a grave general septicæmia with all its possible complications.

(2) These infections may be mixed or secondary, due to the entrance into the circulation of organisms other than the gonococcus, or they may be purely gonococcal in nature.

(3) Endocarditis is an occasional complication of gonorrhœa.

(4) This endocarditis may be transient, disappearing with but few apparent results, or it may leave the patient with a chronic valvular lesion, or it may pursue a rapidly fatal course with the symptoms of acute ulcerative endocarditis.

(5) The endocarditis associated with gonorrhœa is commonly due to the direct action of the gonococcus, but may be the result of a secondary or mixed infection.

(6) Pericarditis may also occur as a complication of gonorrhœa, but it is less frequent than endocarditis. It may, as in the case of the latter, be the result either of a pure gonococcal or of a mixed infection.

(7) Grave myocardial changes, necroses, purulent infiltration, embolic abscesses are common in the severe gonococcal septicæmias.

(8) In instances of gonococcal septicæmia the diagnosis may, in some cases, be made during life by cultures taken from the circulating blood according to proper methods.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(1) Bourdon. *Gaz. d. Hôp.*, 1868, xli, 1.	M	24	Gonorrhœa; one month later, arthritis; delirium; irregular chills; evidences of endocarditis. Hæmorrhagic areas in skin, becoming gangrenous; bed sores. Death 13 months after infection.	Verrucous endocarditis of mitral and tricuspid valves with thrombi. Kidneys pale and fatty.	
(2) Desnos. *L'Union Méd.* 3 s., 1878, xxv, 43. Also *Gaz. d. Hôp.*, 1877, L, 1867.	M	?	Gonorrhœa; bronchitis; arthritis; dyspnœa; palpitation; cardiac murmur; irregular fever. Death less than a month after onset of arthritis. The heart was normal on first examination. No history of any previous predisposing malady.	Vegetative endocarditis of aortic and mitral valves.	In the discussion of the case Fournier acknowledged its gonorrhœal origin.
(3) Schedler. "Zur Casuistik der Herzaffectionen nach Tripper." Inaug. diss. Berlin, 1880.	M	22	Gonorrhœa; epididymitis; arthritis and moderate fever, shortly after onset. 7 months later, irregular chills; evidences of aortic endocarditis with insufficiency. Death 5 weeks after onset of acute symptoms.	Ulcerative endocarditis of aortic valve.	Patient had had smallpox and a fever some time previously, which justifies one in asking whether the endocarditis may not have preexisted. At the autopsy the changes pointed to a recent affection. The reporter as well as Prof. Leyden have no doubt as to its gonorrhœal origin.
(4) Draper. *Medical Bulletin*, Philadelphia, 1882, iv, 81.	M	19	Chronic gonorrhœa for a year; arthritis; irregular fever, with chills; embolism in leg. Death 4 months after onset of arthritis.	Ulcerative endocarditis of mitral valve. Infarctions in spleen and kidneys.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(5) Martin. *Rev. Méd. de la Suisse Romande*, 1882, ii, 308, 352.	M	24	Previous history good. Lost appetite and began to feel ill 3–4 weeks before entrance into hospital. Chills; irregular fever; evidences of pyæmia; multiple arthritis; hæmaturia; right pleuro-pneumonia; suppurative conjunctivitis; parotitis. Death a month after beginning of symptoms.	Gonorrhœa; suppurative prostatitis; cystitis; suppurative inflammation of vesiculæ seminales; multiple myocardial abscesses. Ulcerative and vegetative endocarditis of mitral valve. In the thrombi on valves were numerous cocci, some of which were exactly similar morphologically to Neisser's gonococci. The kidneys showed fatty degeneration with septic emboli.	The case would appear to be of gonorrhœal origin, whether or not a secondary infection occurred.
(6) Fleury. *Journ. de Méd. de Bordeaux*, 1883-'84, xiii, 65.	M	27	Gonorrhœa; 3 months later disappearance of discharge; arthritis; thoracic pains; pericarditis. Death 3 weeks after arthritis. No signs of cardiac disease on examination before this infection.	Vegetative and ulcerative endocarditis of aortic valves with perforation. A certain amount of blood-stained fluid in the pericardium; a patch of false membrane just below the root of the aorta.	
(7) Weckerle. *Münch. med. Woch.*, 1886, xxxiii, 563, 582, 608, 622, 636.	F	24	Gonorrhœa; inguinal adenitis; arthritis; 1 to 2 months after arthritis, signs of ulcerative endocarditis of pulmonary valve; chills; irregular fever; dry pleurisy. Death a month after onset. The urine, normal at first, showed a large quantity of albumin; sediment; red and white corpuscles: renal epithelium and casts.	Sero-fibrinous pericarditis. Extensive ulcerative endocarditis of pulmonary valves. Numerous cocci in the thrombi on the valves, sometimes in chains, sometimes in groups. These organisms stain according to Gram. No particular search was made for gonococci. Kidneys large—subacute parenchymatous nephritis.	The author does not believe the endocarditis to have been gonococcal. The only portal of entry appears to have been furnished by the gonorrhœa.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(8) Weichselbaum *Centralbl. f. Bakt.*, 1887, ii, 209. Also, Ziegler's *Beiträge*, 1889, iv, 125.	M	21	Gonorrhœa for 3 weeks; high fever. Death on day of entry. Gonococci in urethral secretion.	Acute splenic tumor. Ulcerative aortic endocarditis. Gonococci in urethra. Only streptococci, morphologically and by culture, on the valves.	W. believes that the gonorrhœa formed the portal of entry for the streptococcus.
(9) Rothmund. "Endocarditis Ulcerosa." Dissertation, Zürich. 1889.	M	51	Gonorrhœa; 8 or 9 weeks later epididymitis; poly-arthritis; heart sounds clear; moderate fever. 3 weeks later loud systolic murmur over mitral and tricuspid areas. 10 days later, high pitched diastolic murmur over aortic and pulmonary regions; delirium; jaundice. Death 5 weeks after arthritis.	Urethritis; cystitis; right knee joint and left ankle contain sero-hæmorrhagic fluid. Extensive ulcerative endocarditis of aortic valves. In the blood of the right heart and in the affected joints were found cocci having the form and grouping of gonococci.	
(10) Ely. *Med. Record*, 1889, xxxv, 287.	M	28	No history. Ill 3 days. Fever; delirium; vomiting. 2 days after entry partial left hemiplegia and death.	Gonorrhœa; old pericardial adhesions; fresh vegetative and ulcerative endocarditis of mitral valve. In thrombi on valve there were cocci and bacilli. In all embolic abscesses in liver, kidneys and in heart valves as well as in urethra, similar cocci staining by Gram and arranged in clusters and chains, are seen. In urethral pus, gonococci decolorizing by Gram are also seen.	The author believes the case to be one of secondary infection with pyogenic cocci.

REPORTER.	SEX.	AGE	HISTORY.	AUTOPSY.	REMARKS.
(11) His. *Berl. klin. Woch.*, 1892, xxix, 993.	M	19	Gonorrhœa; first attack, disappearing apparently in 3 weeks. 2 weeks later syncopal attack, followed by chills; hæmorrhagic eruption; remittent fever; loud systolic murmur; signs of ulcerative endocarditis. Blood cultures from ear negative.	General anæmia; ecchymoses of skin and serous membranes; ulcerative aortic endocarditis; softened thrombus at cardiac apex. Acute interstitial myocarditis; septic emboli in spleen, kidneys and lungs. In the thrombi on the affected valve cocci resembling gonococci were found; these decolorized when treated according to Gram. Unfortunately, the specimen had been previously put in Müller's fluid.	
(12) His. *Op. cit.*	M	19	Gonorrhœa; arthritis; a few days later irregular fever and chills, sometimes two a day. 4 months after onset of gonorrhœa there were signs of ulcerative endocarditis; aortic stenosis. Death $5\frac{1}{2}$ months after onset of gonorrhœa.	Acute aortic endocarditis; partial aneurism at root of aorta with papillary thrombi; enlarged spleen with fresh infarcts; subacute parenchymatous nephritis; slight hydrothorax; hydropericardium. No bacteriological examination.	Case observed by Wagner in 1879. Autopsy by Huber.
(13) Leyden. *Deutsch. med. Woch.*, 1893, xix, 909.	M	22	Chronic gonorrhœa; epididymitis; arthritis of right knee; signs of ulcerative endocarditis with aortic insufficiency; irregular fever; chills. Death 6 to 7 weeks after onset of arthritis. Cultures from vein on ordinary media, negative.	Acute myocarditis; ulcerative endocarditis of aortic and vegetative endocarditis of mitral valves. Typical gonococci decolorizing by Gram in the thrombi on valves. Cultures on ordinary media negative.	Doubtless a pure gonorrhœal endocarditis.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(14) Wilms. *Münch. med. Woch.*, 1893, xl, 745.	M	26	Gonorrhœa 6 months ago and again later; 3 weeks after second attack, chill, arthritis in knee; a week later signs of aortic insufficiency; high fever. Death 6 weeks after the second infection.	Ulcerative endocarditis of aortic valve extending through into right auricle; suppurative myocarditis. Numerous cocci in thrombus, some separate, some arranged like gonococci and intracellular. They decolorize almost immediately by Gram. In leucocytes in the sub-mucous tissue of urethra there were scanty diplococci, some resembling gonococci.	The author does not believe the organisms were gonococci though the description is more than suggestive.
(15) Golz. "Ulceröse Endocarditis der Klappen der Pulmonalarterie bei gonorrhoischer Arthritis." Inaug. Diss. Berlin, 1893.	M	21	Gonorrhœa; right-sided bubo; 6 days later arthritis in right shoulder and left foot. Gonococci found in urethra. 2 weeks later chills; heart sounds clear; chills and fever continued, and 2 weeks later systolic murmur became audible; diastolic murmur in pulmonary area. 3 weeks later pericarditis and pleurisy with hæmorrhagic exudate; pulmonary embolism. Death 3½ months after infection and a little less than 3 months after apparent onset of endocarditis.	Fibrinous pericarditis; in pericardium ½ litre of fairly clear fluid. Ulcerative endocarditis of pulmonary valve and of wall of right auricle. No cultures. The specimen had been left for a considerable time in alcohol before a bacteriological examination was made. Small cocci at times in groups and sometimes in chains were found. No characteristic gonococci.	The organisms described by the author appear to have been staphylococci and streptococci.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(16) Councilman. *Am. Jour. Med. Sci.*, 1893, cvi, 540.	M	?	Gonorrhœa; 10 days later arthritis in various joints. Five weeks after infection pericarditis. Death suddenly 3 days later.	Pericardium contained 800 cc. of hæmorrhagic exudate in which there were large masses of clot. Both surfaces of pericardium covered with thick membranous masses, containing hæmorrhages. Suppurative myocarditis. Gonococci, characteristic morphologically, in suppurative foci in heart muscle, pericardium, urethra and joints; they decolorized by Gram. No cultures.	
(17) Fressel. Inaug. Diss. Leipsic, 1894.	F	26	History imperfect. Severe symptoms came on a few days before death. Extreme weakness; emaciation; orthopnœa; arthritis of left ankle.	Ulcerative and polypoid endocarditis of mitral and aortic valves. Kidneys practically normal. Cystitis; urethritis; vaginitis. The thrombi on valves showed organisms having microscopically the characteristics of the gonococcus; some occupying cells; they decolorized by Gram's method. No cultures.	
(18) Winterberg. Festschrift z. 25 Jähr. Jub. des Vereins Deutscher Aerzte zu San Francisco, 1894, 40.	M	25	Gonorrhœa; 6 weeks later right epididymitis; double bubo; arthritis of both elbows; dyspnœa; systolic and diastolic murmurs especially in aortic area. Death after onset.	Pleural effusion on both sides; 20 cc. sero-purulent fluid in pericardium; myocardial abscesses. Ulcerative endocarditis of aortic and pulmonary valves which were almost entirely destroyed. Endocarditis of mitral and tricuspid valves of a lesser extent. Amyloid kidneys. No cultures. Cover glass specimens from valves showed organisms answering morphologically and tinctorially to gonococci, decolorizing by the method of Gram.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(19) Thayer and Blumer. *Arch. de Méd. Expér.*, 1895, vii, 701, and Johns Hopkins Hospital Bull., 1896, vii, 57.	F	34	Vague history of rheumatism 3 months ago; has been short of breath 3 or 4 years. About 3 months after "rheumatism," irregular fever and chills; signs of ulcerative endocarditis; mitral stenosis. Death 3 weeks after onset of fever. Cultures from median basilic vein 9 and 4 days before death showed pure growths of diplococci resembling in every way gonococci and decolorizing by Gram's method. The medium contained at least one-third blood (the syringe full of blood mixed with agar agar and plated). Transplanted to ordinary media there was no growth.	Ulcerative endocarditis of mitral valve. In the thrombi on valves and in vagina and uterus characteristic intracellular gonococci, decolorizing when treated according to Gram's method. Cultures made on agar agar and bullock's blood serum from all sources were negative.	This is the first case in which gonococci were obtained in pure culture from the blood during life.
(20) Finger, Ghon and Schlagenhaufer. *Arch. f. Dermat. u. Syph.*, 1895, xxxiii, 141, 323.	M	19	Chronic gonorrhœa for a year. Fresh attack in March, 1895. 6 months later arthritis in right knee; fever. Heart sounds clear; gonococci in urethral discharge; chills; 10 days later diastolic murmur over aorta, Death 9 days later.	Myocarditis; ulcerative aortic endocarditis with perforation. Arthritis of right knee joint. Chronic urethritis; prostatic abscess; infarct of spleen; cloudy swelling of kidneys. Characteristic gonococci in urethra and in thrombi on valves, decolorizing by Gram. Cultures on ox's serum peptone agar were negative excepting from urethra, from which an unidentified coccus was obtained.	The authors believe the case to be purely gonorrhœal, the gonococci having lost their vitality, possibly owing to high temperature before (and after, W. S. T.,) death, and hence failing to grow.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(21) Zawadzki and Bregman. *Wien. med. Woch.*, 1896, xlvi. 313, 351.	F	17	A month ago purulent vaginitis with staphylococcus and micrococcus tetragenus; chills and fever; 6 weeks after infection, arthritis in hip not yielding to salicylates; 11 days later, serous pleurisy on right side, showing on culture, streptococcus pyogenes; staphylococcus albus; micrococcus tetragenus. 15 weeks after onset right hemiplegia and death.	Verrucous mitral endocarditis; embolism of right arteria fossæ Sylvii. In the excrescences on valves among other organisms were numerous characteristic gonococci. These were in part in groups in the intermediate tissue, in part in the cells and in their neighborhood. They had the character and shape of gonococci and were decolorized when treated according to Gram. They were more numerous on the free border of the valves as were the other cocci.	A mixed infection. Some question as to whether the organisms found were gonococci.
(22) Dauber and Borst. *Deutsch. Arch. f. kl. Med.*, 1896, lvi, 231.	M	20	Gonorrhœa; tenosynovitis of left hand; inguinal buboes; periurethral abscesses. 2 weeks after onset, chill; irregular fever; heart's sounds clear. Pain in cervical vertebræ. 3 months after infection evidences of aortic insufficiency; septic nephritis. Gonococci gradually disappeared from urethral discharge, other bacteria appearing. Cultures from blood, negative. Death.	Ulcerative and vegetative endocarditis of aortic valve; suppurative myocarditis; acute nephritis; septic emboli and infarctions of kidney. In thrombi organisms showing all the characteristics of gonococci, decolorizing by Gram; also once in colorless corpuscle in heart's blood. Cultures negative excepting one on human blood serum agar on which there developed after 36 hours separate point-like, yellowish brown translucent colonies. On feeble magnification these appeared round, had a sharp clean-cut border, showed no outgrowths from their peripheries or daughter colonies so characteristic of gonococci. Micro-organisms obtained from the culture were almost entirely diplococci, in part biscuit shaped, in part, round.	While the authors doubt that these were gonococci, most will probably accept the case as positive. Vide Michaelis, *Op. cit.* and Thayer and Blumer. *Johns Hopkins Hospital Bulletin*, 1896, vii, 57.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(23) Michaelis. *Zeitschr. f. kl. Med.*, 1896, xxix, 556.	M	25	Gonorrhœa; 3 weeks later, arthritis; slight fever; no albuminuria; 4 days later, systolic murmur over aorta; diastolic sound not clear. Sudden death 10 days later. Gonococci in urethral discharge.	Vegetative and ulcerative aortic endocarditis with perforation. Pericardial and pleural cavities showed abundant serous fluid with a few fibrinous flakes. Purulent cystitis. Characteristic gonococci in thrombi on valves, i. e. shape; intracellular arrangement; decolorization by Gram's method; failure to grow on attempts to cultivate on ordinary media.	In all probability a pure gonococcal endocarditis.
(24) Keller. *Deutsch. Arch. f. kl. Med.*, 1896, lvii, 387.	M	25	Gonorrhœa; arthritis 4 weeks after; later pains in chest; chills; irregular intermittent fever; evidences of pulmonary endocarditis; pericarditis. Death 4 months after infection and 3 months after arthritis. Diagnosis: pulmonary stenosis and insufficiency.	Increase in pericardial fluid which was made cloudy by the presence of fine flocculi. Vegetative endocarditis of pulmonary valves and pulmonary artery; myocarditis. Streptococci in cultures from pericardial fluid. In polypi on pulmonary valve streptococci were found; streptococci in kidneys.	The author believes that this was a mixed infection through the urethra.
(25) Hale White. *Lancet*, 1896, i, 533.	M	19	Gonorrhœa; 3 weeks later, chills; irregular intermittent fever; anæmia; systolic and diastolic murmurs in pulmonary area; 5 weeks after infection, acute nephritis; œdema. Two weeks later, death.	Ulcerative and vegetative endocarditis of the pulmonary valve and artery. Acute nephritis; characteristic gonococci in vegetations on valves. These decolorized when treated by Gram's method. Cultures taken on agar, glycerine agar, broth and blood serum were without result. No cultures were made on serum agar. (These latter particulars were obtained in a personal communication from Dr. Pakes.)	The author remarks on the frequency of nephritis in ulcerative endocarditis, and believes it to be a not uncommon cause of death.

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(26) Babes and Sion. *Arch. d. Sc. Méd. de Bucharest*, 1896, i, 505.	M	?	August 5, gonorrhœa; 20 days later cystitis; epididymitis; purpura; fever; chills; vomiting; splenic tumor. On September 2, aortic systolic murmur; albuminuria; diarrhœa. Death October 14. In blood during life Opresco saw cocci resembling gonococci and decolorizing by Gram; they were within leucocytes.	Gangrene of skin over lower abdomen and genitalia; hæmorrhagic infarct of kidney; ulcerative aortic endocarditis. In the thrombi on valves organisms similar to gonococci were found, decolorizing by Gram. On ordinary media saprophytes alone grew. No growths on beef blood serum agar. In spleen and kidney strepto- and staphylococci.	They believe the case to have been gonorary streptococcus and rhœal with a second staphylococcus infection. They seem to think that, the pus cocci having entered and caused a general septicæmia, the gonococci profiting by the diminished resistance of the organism entered later and attacked the valves.
(27) Stengel. *Univ. Med. Mag.*, Phila., 1897, ix, 426.	F	20	Had had valvular heart disease, since rheumatism, at age of 7; gonorrhœa for a year (?); 6 days before entry into hospital diarrhœa; headache, vomiting, tympanites; evidences of mitral stenosis; acute nephritis. Continued fever. Death after 32 days.	Chronic endocarditis of mitral valve. Fresh ulcerative and vegetative endocarditis of mitral valve and part of auricle, particularly marked on an anomalous adventitious leaflet. Muco-purulent exudate in uterus and vagina. Cultures: Streptococcus from lungs and staphylococcus from right auricle and spleen; endocardial vegetations negative. Characteristic gonococci were found in two thrombi on valves, mainly intracellular, decolorizing by Gram. No gonococci found in exudate in uterus or vagina. Deeper tissues not examined.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(28) Thayer and Lazear. Subject of present communication, read before XII International Congress in Moscow, 1897. *Med. Record*, 1897, lii, 497.	M	19	Gonorrhœa 6 months before entry into hospital; several weeks later chill; 5 to 6 months later œdema of legs; subacute nephritis; grave anæmia; systolic murmur; pericarditis. Death 7½ months after infection, about 6 months after onset of chills. Gonococci obtained three times during life from circulating blood. Diagnosis: gonorrhœal septicæmia, endo- and pericarditis.	Ulcerative and vegetative endocarditis of tricuspid valve. Sero-purulent pleurisy and pericarditis; subacute hæmorrhagic and glomerular nephritis. Gonococci obtained microscopically and in pure culture on Loeffler's blood serum agar from heart's blood, pericardium and affected valves. All other cultures negative.	This is the first case in which gonococci were obtained in pure culture before and after death from the blood, thus permitting a positive ante-mortem diagnosis of gonorrhœal septicæmia.
(29) Rendu and Hallé. *Bull et. mém. Soc. méd. de hôp. de Par.* 1897, 3 s., xiv, 1325.	F	30	Gonorrhœal metritis; about 2 weeks later fever; night sweats; evidences of septicæmia. Gonococcus isolated from uterine mucus. Phlegmon near elbow joint; intermittent fever; endo- and pericarditis about 5 weeks after onset of fever. Death 10 days later. In phlegmonous œdema at elbow gonococci "à l'état de pureté" were found. Blood cultures negative.	Vegetative endocarditis of aortic valve and of ascending aorta. Sero-fibrinous pericarditis. Cultures from pleural and pericardial fluids negative. The bacteriological and histological examination of the aortic vegetations showed the exclusive presence of gonococci.	Were the gonococci obtained in pure culture?
(30) Siegheim. *Ztschr. f. kl. Med.* 1898, xxxiv, 526.	F	20	Chills and fever and systolic murmur in tricuspid area in June; later, systolic murmur in mitral, and diastolic in aortic area; dyspnœa; irregular intermittent fever; chills. Death July 11. Cultures from blood on Kiefer's agar agar and peptone bouillon were negative.	Ulcerative endocarditis of aortic valve; myocarditis; nephritis; purulent endometritis and cystitis. Cultures on Kiefer's agar from heart's blood and vegetations negative. Microscopical examination of smear preparations from thrombi revealed organisms showing all the morphological and tinctorial characteristics of gonococci.	

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
(31) Lenharz. *Berl. kl. Woch.*, 1897, xxxiv, 1138.	F	19	Abundant vaginal discharge; symptoms of ulcerative endocarditis of pulmonary valve. Death.	Vegetative endocarditis of pulmonary valves. Characteristic gonococci in softened thrombi. These were obtained in pure culture. A piece of softened thrombus was introduced into the human urethra resulting in the development, after four days, of gonorrhœa with typical organisms.	This case would appear to remove all doubt as to the possibility of the existence of a true gonorrhœal endocarditis.
(32) Unpublished observation from the wards of Prof. Osler, Johns Hopkins Hospital. (Case observed by one of the authors—Thayer).	M	38	Measles as a child; no other acute infectious diseases. Entered hospital 20/vii/94. Has gonorrhœa with characteristic gonococci in discharge. For 3 weeks irregularly intermittent fever with chills. *Physical examination:* negative; heart sounds clear; apex impulse in 5th interspace, just inside mammillary line. Leucocytes 17,000 per cu. mm.; *urine* shows trace of albumin; no casts found. Irregular fever with severe rigors; left hospital unimproved on 2 / viii / 94. Urethral discharge and rigors stopped two weeks later; fever however continued. A few days after leaving hospital, stabbing pains in præcordial region, somewhat relieved by pressure over heart; dyspnœa. In latter part of October puffiness of eyelids, frequency of	(By Dr. Flexner.) Chronic and acute endocarditis (vegetative and ulcerative) of the pulmonary valves; deficiency of one segment; vegetation extending 3 cm. into the pulmonary artery; globular thrombi in left ventricle; acute pneumonia; chronic diffuse nephritis. Coverslips from the thrombi on the affected valve show encapsulated diplococci; others biscuit shaped, resembling gonococci. On treatment by Gram's method some apparently decolorized, others did not. Characteristic gonococci in urethra. Cultures on ascitic fluid agar and Loeffler's blood serum from thrombi on affected valve, mitral valve, heart's blood, pleura, lung, kidney, spleen, liver and bladder all showed pneumococci.	The clinical history suggests strongly that the original process was associated with the gonorrhœa, while the pneumococcus infection was a late secondary event. The following note was made by Dr. Flexner: "Note.— Had the gonococcus been present it is probable that it would have been so obscured by the universal presence of the the Diploc. pneumoniæ that it could not have been detected."

REPORTER.	SEX.	AGE.	HISTORY.	AUTOPSY.	REMARKS.
			micturition, purpuric eruption on legs. Re-entered hospital 7/xi.—Physical examination: puffiness of face; pulse of high tension. Heart: apex impulse in 5th space 2 cm. outside nipple; slight to-and-fro murmur over pulmonary orifice, so superficial that it was believed to be pericardial; purpuric eruption on legs and thighs. *Urine:* trace of albumin; sediment: hyaline, granular, pus casts and renal epithelial cells. *Blood:* 13/xi; red corpuscles, 3,920,000; colorless, 31,200; hæmoglobin, 46%. 14/xi; diastolic murmur, heard by Prof. Osler in pulmonary area. Irregular fever disappeared on the 18th and T° was subnormal afterwards, excepting on the 21st, 22nd and 23rd. The dyspnœa increased, pneumonia developed, and on 26/xi the patient died. The day before death the leucocytes were 78,000 per cu. mm. Cultures from the blood, made by Dr. Blumer on 17/xi, were negative.		

ON ALTERATIONS IN THE COMPOSITION OF THE BLOOD RESULTING FROM EXPERIMENTAL DOUBLE NEPHRECTOMY.

By C. A. HERTER, M. D., and A. J. WAKEMAN, Ph. D.

Although the alterations in the composition of the blood which follow uncomplicated experimental double nephrectomy are of much interest in relation to the pathology of human uræmic states, the literature relating to the subject is exceedingly limited, and it is certain that investigators have not given it the attention of which it is worthy. It is the aim of this paper to present concisely the results of experimental work relating chiefly to the chemical changes that occur in the blood as the result of double nephrectomy. In most instances dogs were employed as the subjects of these observations, and both kidneys were removed at one operation. The chief symptoms following the removal of both kidneys in dogs are as follows: lowering of the temperature, loss of appetite, drowsiness, vomiting, diarrhœa.

In a few instances the second kidney was removed several days after the removal of the first. In a smaller series of cases studies were made of the blood after both ureters had been tied just below the pelves of the kidneys. In almost all cases the animals were carefully watched, and were bled to death as soon as there were indications that life was not likely to be prolonged for many hours. This method of procedure is of importance, because there is reason to think that the composition of the blood undergoes alterations in certain respects by contact post mortem with the tissues.*

Thus v. Limbeck obtained the following results:

	NaCl.		KCl.		HNa_2PO_4	
	During life.	48 hours post mort.	During life.	48 hours post mort.	During life.	48 hours post mort.
Blood.....	.1643	.1642	.0279	.0567	.0247	.0327
Muscle.....	.0193	0.198	.3261	.2271	.3286	.2721

* v. Limbeck, *Arch. f. exp. Path. u. Pharm.*, 1892, xxx, 195.

Our observations have reference to the reaction of the blood and to its content of urea, of uric acid, of alcoholic and ethereal extractive substances, of ethereal extractive substances, of total proteids, of fibrin, of ash, of sodium and potassium, and of phosphoric acid.

*Reaction of the blood.**—Reference to Table I shows that the alkalinity of the blood was distinctly increased as the result of double nephrectomy. Thus the average alkalinity in six normal dogs was .0043 grm. NaOH (1 cc. blood), while that of six nephrectomized dogs was .0095 grm. NaOH. In the case of two dogs whose ureters had been tied, the alkalinity gave an average of .0138 grm. NaOH, but these results do not of course justify the conclusion that a higher grade of alkalinity is to be regularly expected after ligation of the ureters than after nephrectomy.

TABLE I.—Alkalinity of Blood.

No.	Nature of experiment.	No. of hours.	1 cc. blood in terms of cc. of $\frac{1}{10}$ normal tartaric acid sol.	1 cc. blood in terms of grammes of NaOH.	Normal dog's blood. 1 cc. blood in terms of cc. of $\frac{1}{10}$ normal tartaric acid sol.	Normal dog's blood. 1 cc. blood in terms of grammes of NaOH.
1	Double Nephrectomy.	48	4.10 cc.	.0164 grms.	.940	.0037 grms.
2	"	52	1.65 "	.0066 "	.950	.0038 "
3	"	52	2.91 "	.0116 "	.940	.0037 "
4	"	52	2.21 "	.0088 "	.730	.0029 "
5	"	72	1.44 "	.0057 "	1.05	.0042 "
6	"	72	1.93 "	.0077 "	1.90	.0076 "
7	Both ureters ligated.	48	3.10 "	.0124 "		
8	"	52	3.80 "	.0152 "		

Average of results, Nos. (1–6) = 2.37 cc. = .0095.
" " " " (7–8) = 3.45 cc. = .0138.
" " " " (1–8) = 2.64 cc. = .0106.
" " " " Normal = 1.08 cc. = .0043.

This increased alkalinity after nephrectomy is especially noteworthy, as it contrasts with the well-known results of von Jaksch in

* *Method:* To a given amount of freshly drawn blood a tenth normal solution of tartaric acid containing sodium sulphate is added. The point of neutralization is determined by the use of litmus paper. For the details of the process see Hoppe-Seyler's Handbuch d. physiol.- u. path.-chem. Analyse, p. 399, Berlin, 1893. The method for absolute values is far from accurate on account of the difficulties involved in determining the neutral point, but is probably sufficiently accurate to render relative results trustworthy.

human uræmia.† It is very doubtful if it can be ascribed entirely to loss of water, as the increase in the specific gravity of the blood is inconsiderable.

*Urea.**—The increase in the percentage of urea in the blood is perhaps the most striking alteration in the composition of the blood after double nephrectomy (Table II). Twenty-eight observations on the blood of normal dogs give an average percentage of .037. Eighteen observations on nephrectomized dogs (which lived from 22 to 82 hours) yield an average of .315 per cent, while six observations on the blood of dogs whose ureters were tied give an average of .301 per cent. The increase in the percentage of urea thus amounts to nearly 10 times the average normal percentage in the course of 82 hours or less. The highest individual percentage in the blood was .458, or nearly .5 per cent. A similar increase in the urea of the blood has been observed by us in several cases of uræmia. It is noteworthy that the average normal percentage of urea in human blood is about the same as that of dogs, and that the highest percentages of urea which have been found in human uræmia are comparable with the highest noted after nephrectomy.

The urea which is stored in the blood after removal of the kidneys is derived from the waste of proteid tissue, and perhaps in a measure directly from proteid food in the digestive tract at the time of opera-

† v. Jaksch. *Zeitschr. f. klin. Med.*, xiii, 350. Further work is certainly needed to determine whether the diminished alkalescence of the blood observed by von Jaksch is actually a feature of the blood in human uræmia.

* *Method:* A given quantity of blood is treated with four or five times its volume of absolute alcohol and allowed to stand 24 hours. The filtrate and washings are evaporated to dryness at a moderate temperature, and the residue is taken up in absolute alcohol and filtered. The filtrate and washings are again evaporated to dryness, and the residue dissolved in a little water. This solution is subjected to the action of sodium hypobromite, and from the volume of nitrogen evolved is calculated the percentage of urea. Other nitrogenous alcoholic extractives of blood, as creatin and lecithin, are partly decomposed with liberation of nitrogen in this process, and herein lies the inaccuracy of the method. Urea, however, is by far the most abundant of the nitrogenous extractives, and the only one which is wholly broken up by the action of sodium hypobromite. The results obtained by this method are looked upon as sufficiently accurate for the purposes of this investigation.

tion.* After operation the animals seldom eat, and the formation of urea is probably little greater than in a state of starvation. The waste in starvation is, however, considerable. A dog weighing 12 kilos, and possessing, say, one kilo of blood, would produce enough urea to cause an accumulation of .5 per cent of urea in the blood in the course of three days, supposing the formation of urea to be even less than 2 grammes daily and further supposing the blood to be entirely free from urea at the time of operation. The daily formation of urea in a starving dog weighing 12 kilos is, however, considerably greater than 2 grammes daily. If all the urea formed were stored in the blood, the percentage would probably rise to 1 in three days. The discrepancy between the amount which should theoretically be found and the amount actually stored in the blood can probably be explained wholly by the fact that after nephrectomy urea

TABLE II.—Urea in the Blood.

No.	Nature of experiment.	No. of hours.	Percentage of urea.	Normal dog's blood. Percentage of urea.	No.	Nature of experiment	No. of hours.	Percentage of urea.	Normal dog's blood Percentage of urea.
1	Double nephrectomy	22	.394	.049	19	Both ureters ligated	48	.260	.027
2	"	26	.256	.040	20	"	36	.342	.058
3	"	36	.103	.035	21	"	45	.200	.021
4	"	40	.121	.023	22	"	48	.323	.063
5	"	48	.350	.023	23	"	52	.302	.022
6	"	48	.395	.026	24	"	72	.377	.011
7	"	48	.276	.021	25	Left kidney removed, right ureter ligated.	24	.254	.013
8	"	48	.184	.034					
9	"	48	.347	.070					
10	"	52	.259	.036					
11	"	52	.366	.079	26	Right ureter ligated, left renal ligated.	36	.247	.036
12	"	52	.286	.073					
13	"	60	.431	.042					
14	"	60	.386	.025					
15	"	72	.458	.031	27	Both renals ligated.	36	.330	.027
16	"	72	.456	.045					
17	"	72	.220	.027	28	Both renals ligated, both ureters ligated.	36	.310	.027
18	"	82	.377	.049					

Average of results: Nos. (1–18) = .315.
" " " (19–24) = .301.
" " " (1–28) = .308.
" " Normal = .037.

* Chittenden. On Digestive Proteolysis, p. 105, New Haven, 1895.

accumulates in the muscles, brain, and other tissues and organs. A similar storage in the tissues is noticeable both in human uræmia and after the experimental intravenous injection of a solution of pure urea in dogs, and makes the percentage of urea in the blood lower than it would be were all the urea retained in the blood.

*Uric acid.**—The few observations upon uric acid recorded in Table III indicate that the salts of uric acid are not increased after nephrectomy—the observations, however, are too few to be conclusive.

If it be a fact that uric acid occurs in minute quantities in normal blood and that it is not increased after nephrectomy, this is of considerable physiological interest. Speculations on the possible reasons for the absence of an increase are of little interest until the fact itself is established.

TABLE III.—Uric Acid in the Blood.

No.	Nature of experiment.	No. of hours.	Percentage of uric acid.	Remarks.	Normal dog's blood. Percentage of uric acid.	Remarks.
1	Double nephrectomy.	48	.0016	Residue did not respond to murexide test.	.0022	Residue did not respond to murexide test.
2	Both ureters ligated.	48	.0025	" "	.0014	" "
3	" " "	52	.0024	" "		

Average of results: Nos. (1 to 3) = .0022.
" " " Normal = .0018.

Alcoholic and ethereal extractives.†—A moderate increase in the alcoholic and ethereal extractives appears to be a feature of the blood after removal of both kidneys. Thus the average of fourteen determinations on the blood of nephrectomized dogs gives 1.29 per cent, while the average of thirteen results obtained from the study of

* *Method:* After the removal of the proteids in the well-diluted blood by heat and acetic acid, the method employed was essentially that of Salkowski modified by von Schroeder. See Hoppe-Seyler's Handbuch d. physiol.- u. path.-chem. Analyse, p. 398, Berlin, 1893.

† *Method:* A given quantity of blood is treated with a large excess of alcohol and allowed to stand. The precipitate is washed with hot absolute alcohol, and afterwards extracted with ether. The alcoholic filtrate and washings are evaporated and the residue extracted and washed with cold and hot absolute alcohol. The combined alcoholic and ethereal residues are dried at 105° C. and the ash deducted.

normal dog's blood gives .95 per cent (Table IV). A similar moderate increase is often observed in the blood of fully developed human uræmia, and also in the blood in some infectious conditions which do not give the typical clinical evidences of uræmia. The alcoholic and ethereal extractives include in larger or smaller quantities fats, cholesterin, lecithin, creatin, urea and salts of hippuric acid, sarco-lactic acid and carbamic acid, sugar and xanthin bodies.

TABLE IV.—Alcoholic and Ethereal Extractives of the Blood.

No.	Nature of experiment.	No. of hours.	Percentage alcoholic and ethereal extractives.	Normal dog's blood. Percent. alc. and eth. extractives.
1	Double nephrectomy.	36	1.02	1.11
2	" "	40	1.33	1.10
3	" "	48	1.13	1.33
4	" "	48	1.41	1.03
5	" "	48	.97	.88
6	" "	48	1.13	.83
7	" "	48	1.31	.98
8	" "	52	1.26	.85
9	" "	52	1.54	.82
10	" "	52	1.00	.88
11	" "	60	1.72	.80
12	" "	72	1.13	.80
13	" "	72	1.31	.94
14	" "	82	1.76	
15	Both renals ligated.	36	1.76	
16	Both ureters ligated.	45	1.10	

Average of results: Nos. (1–14) = 1.29.
" " " (1–16) = 1.30.
" " Normal. = .95.

*Ethereal extractives.**—The percentage of increase in the ethereal extractives is greater than that of the combined alcoholic and ethereal extract. It is seen in Table V that the average of eight results obtained from the blood of dogs whose kidneys had been removed or ureters tied is .299, which is more than double the average result obtained from the study of the blood of six normal dogs. The

* *Method:* A known weight of blood is dried and a given quantity of the finely powdered residue is thoroughly extracted in Soxhlet's extraction apparatus. The extract is dried at 105° C. and weighed and the ash deducted.

ethereal extractives of blood include neutral fats and cholesterin, as well as lecithin and salts of hippuric acid, and possibly of sarcolactic and carbamic acids.

TABLE V.—Ethereal Extractives of the Blood.

No.	Nature of experiment.	No. of Hours.	Percentage of ethereal extractives.	Normal dog's blood. Percentage of ethereal extractives.
1	Double neprectomy.	40	.378	.206
2	" "	48	.308	.104
3	" "	52	.295	.144
4	" "	52	.320	.180
5	" "	72	.358	090
6	" "	72	.130	.100
7	Both ureters ligated.	48	.270	
8	" " "	52	.330	

Average of results: Nos. (1-6) = .298.
" " " " (1-8) = .299.
" " " Normal = .137.

*Total proteids.**—The total proteids of the blood in six normal dogs yielded an average of 6.95 per cent of the constituents of the blood (Table VI). The average percentage of the total proteids in seven

TABLE VI.—Total Proteids of the Blood.

No.	Nature of experiment	No. of hours.	Percentage of total proteids.	Normal dog's blood Percentage of total proteids.
1	Double nephrectomy.	26	6.49	7.07
2	" "	48	9.54	7.44
3	" "	52	6.61	6.90
4	" "	52	8.82	5.10
5	" "	52	7.40	7.11
6	" "	72	7.38	8.09
7	" "	72	7.39	
8	Both ureters ligated.	45	6.55	
9	" " "	48	8.19	
10	" " "	52	6.40	

Average of results: Nos. (1- 7) = 7.66.
" " " (8-10) = 7.05.
" " " (1-10) = 7.48.
" " Normal = 6.95.

* *Method:* The proteids are precipitated in large excess of alcohol. After standing they are brought upon a weighed filter paper, washed with hot alcohol and ether, dried, weighed and the ash deducted.

cases of double nephrectomy was 7.66 per cent and 7.05 per cent in the case of three dogs in which double ligation of the ureters was practised. The inference seems proper that the percentage of the total proteids remains unaltered both after double nephrectomy and after double ligation of the ureters.

*Fibrin.**—The average fibrin yield of the blood of eleven normal dogs was .246 per cent. The average yield from seven nephrectomized dogs was .513 per cent. The average yield from three dogs in which the ureters had been tied was .483 per cent (Table VII). It is not possible at present to account satisfactorily for this apparently constant increase in the fibrin content of the blood after double nephrectomy. At first the possibility suggested itself that the increase might be the result of the simple elimination of the renal functions, but control observations have failed to establish this idea. In some of our operations there was moderate hemorrhage. It is known that considerable hemorrhage is followed by an increase in the fibrin content of the blood, and the question arose whether the increase of fibrin after nephrectomy might not depend on hemorrhage. Several operations were then done with great care to avoid hemorrhage. The fibrin content of the blood in these cases was as high as in the case of the dogs previously nephrectomized. As various infections are associated with an increase of fibrin, and as streptococcus infection occurred in some of our dogs, it seemed possible that our high fibrin results were due to infection. But this explanation would hold true of only a few of our cases; the fibrin content of the blood was increased even in cases where infection could be excluded. It was further found that if both hind legs of rabbits were removed at the knee, the fibrin of the blood was distinctly increased on the third day, even when hemorrhage and infection were absent. Thus from one experiment of this nature the fibrin yield was .702 per cent, and from another .744 per cent, while the average fibrin yield of the blood of eight normal rabbits was .255 per cent. On the other hand a simple

* *Method:* The fibrin estimations were made by the method of Hoppe-Seyler, see Handbuch, p. 410. The fibrin, separated by beating the freshly drawn blood, is thoroughly washed with a dilute solution of sodium chloride, then with water, and finally with boiling alcohol. It is dried at 105° C. and weighed. Duplicate analyses gave uniformly concordant results.

laparotomy on a dog was not followed by any change from the average fibrin content. On the whole it seems more likely that the increase in the fibrin of the blood after nephrectomy is in some obscure way connected with the operation to which the animal is subjected rather than to the elimination of the renal functions *per se.*

TABLE VII.—FIBRIN FROM THE BLOOD.

No.	Nature of experiment.	No. of hours.	Percentage of fibrin.	Normal dog's blood. Percentage of fibrin.
1	Double nephrectomy.	48	.457	.201
2	" "	48	.430	.162
3	" "	52	.585	.221
4	" "	52	.450	.375
5	" "	52	.491	.320
6	" "	72	.690	.320
7	" "	72	.490	.185
8	Both ureters ligated.	45	.600	.206
9	" " "	48	.530	.279
10	" " "	52	.320	.240
				.200

Average of results: Nos. (1– 7) = .513.
" " " (8–10) = .483.
" " " (1–10) = .504.
" " Normal = .246.

*Ash.**—By referring to Table VIII it is seen that the average of eight estimations of the ash from the blood of nephrectomized dogs

TABLE VIII.—ASH OF THE BLOOD.

No.	Nature of experiment.	No. of hours.	ercentage of ash	Normal dog's blood. Percentage of ash.
1	Double neprectomy.	40	.74	.93
2	" "	48	.91	.83
3	" "	48	.90	.94
4	" "	52	.96	.48
5	" "	52	.93	.92
6	" "	52	.72	.64
7	" "	72	.84	
8	" "	72	1.02	
9	Both ureters ligated.	45	1.09	
10	" " "	48	.99	

Average of results: Nos. (1-8) = .88.
" " " " (9-10) = 1.04.
" " " " (1-10) = .91.
" " " Normal = 79.

* *Method:* A given quantity of blood is dried and decarbonized with access of air over a Bunsen burner; the residue is weighed.

is .88 per cent, while the average of six estimations from the blood of normal dogs is .79 per cent. These results would seem to indicate an increase of the non-volatile products of the blood in the case of nephrectomized animals. The difference, however, is so slight, and the observations so few, that one is not justified in attaching much importance to the suggestion.

TABLE IX.—Sodium and Potassium in the Blood.

No.	Nature of Experiment.	No. of Hours.	Percentage of Na_2O.	Normal dog's blood Percentage of Na_2O.	No.	Nature of experiment.	No. f hours.	Percentage of K_2O.	Normal dog's blood Percentage of K_2O.
	Double ne-					Double ne-			
1	phrectomy.	36	.324	.280	1	neprectomy.	36	.067	.015
2	" "	40	.156	.273	2	" "	40	.028	.026
3	" "	48	.213	.273	3	" "	48	.028	.020
4	" "	48	.345	.240	4	" "	48	.027	.036
5	" "	52	.330	.284	5	" "	48	028	.022
6	" "	52	.365		6	" "	48	.034	.015
7	" "	52	.260		7	" "	52	.021	.017
8	" "	60	.181		8	" "	52	.031	.029
9	" "	72	.305		9	" "	52	.033	.027
10	" "	72	.285		10	" "	60	.030	
	Both ureters				11	" "	72	.032	
11	ligated.	45	.396		12	" "	72	.035	
12	" "	48	.392		13	" "	72	.031	
13	" "	52	.418			Both ureters			
					14	ligated.	45	.029	
					15	" "	48	.036	
					16	" "	52	.038	
					17	" "	48	.027	

Average of results: Na_2O, Nos. (1-10) = 276.
" " " Na_2O, " (11-13) = .401.
" " " Na_2O, " (1-13) = .305.
" " " Normal, Na_2O = .270.
" " " K_2O, Nos. (1-13) = .033.
" " " K_2O, " (14-17) = .033.
" " " K_2O, " (1-17) = .033.
" " " Normal, K_2O .023.

*Sodium and potassium.**—The blood from ten nephrectomized dogs gave an average of .276 per cent Na_2O, and three dogs whose ureters had been tied yielded an average of .401 per cent Na_2O (Table IX). Estimations on the blood of five normal dogs showed

* *Method:* A given quantity of blood is carbonized in presence of $(NH_4)_2SO_4$. The residue is extracted with water, and a mixture of $BaCl_2$ and $Ba(OH)_2$ added. The barium is thrown out of the filtrate with $(NH_4)_2CO_3$. The method of procedure is as usual. The weight of the combined chlorides of sodium and potassium is obtained, and the potassium alone is weighed as K_2PtCl_6.

an average of .270 per cent Na_2O. It is thus seen that the sodium content of the blood of nephrectomized dogs is about the same as that found in normal animals. The percentage of sodium in the blood of animals whose ureters had been tied is seen to be considerably higher than the normal, but more observations are needed to determine whether this is regularly the case.

As regards the potassium content of the blood, there is found a slight increase in the case of dogs whose kidneys have been removed or ureters ligated. Thus the average yield from the study of the blood of thirteen nephrectomized dogs is found to be .033 per cent K_2O, and from four dogs whose ureters had been tied the value is the same. The blood of nine normal dogs yielded an average of .023 per cent K_2O.

TABLE X.—Phosphoric Acid in the Blood.

No.	Nature of experiment.	No. of hours.	Percentage of P_2O_5.	Normal dog's blood. Percentage P_2O_5.
1	Double nephrectomy.	48	.100	.111
2	" "	52	.134	.068
3	" "	52	.144	.064
4	" "	72	.115	.063
5	" "	72	.145	
6	Both ureters ligated.	45	.066	
7	" " "	48	.081	
8	" " "	52	.077	

Average of results: Nos. (1–5) = .128.
" " " (6–8) = .075.
" " " (1–8) = .106.
" " Normal = .076.

*Phosphoric acid.**—The study of the blood of five nephrectomized dogs yielded an average of .128 per cent P_2O_5. From three dogs whose ureters had been tied an average of .075 per cent P_2O_5 was obtained. The average from the study of the blood of four normal dogs was found to be .076 per cent P_2O_5 (Table X). These results

* *Method:* A weighed amount of blood is decarbonized by aid of HNO_3. The phosphates in HNO_3 are precipitated with molybdic acid mixture. The precipitate is filtered off and washed and dissolved in $(NH_4)OH$. The phosphates are again precipitated with "Magnesia Mixture," and finally weighed as $Mg_2P_2O_7$.

suggest an increase of the salts of phosphoric acid in the blood of nephrectomized dogs—a suggestion borne out by the study of the potassium content of the blood, as well as the ash, before and after nephrectomy.

Muscle, liver and brain.—The results recorded in Table XI represent the percentage of nitrogen obtained by the action of sodium hypobromite in alkaline solution upon the alcoholic extractives of the various organs.*

In the case of the muscle it is seen from the average of a number of observations that the nitrogen evolved by the action of sodium hypobromite upon the alcoholic extractives after nephrectomy is four to five times as great as that obtained from the alcoholic extractives of normal dog's muscle. A still greater increase is noticeable in the case of the liver. Thus the alcoholic extractives of the liver after nephrectomy liberate about nine times as much nitrogen as the alcoholic extractive of the normal liver. The few observations on the brain show the same marked increase.

While no attempt was made to establish the identity of the substance or substances which contribute to the increased liberation of nitrogen after nephrectomy, it is fair to suppose that the increase was due to the accumulation of urea. This view is borne out by the following experiment. Into the femoral vein of a dog weighing 43 pounds, whose kidneys had been removed, 220 cc. of a 25 per cent urea solution were injected at the rate of 20 cc. per minute. The animal was bled to death immediately after injection and the muscle, freed from blood, gave .066 per cent N when the alcoholic extract was acted upon by sodium hypobromite. Muscle cut from the animal before injection gave .036 per cent N. The increase here is clearly due to urea, since the other muscle extractives during the short time involved would remain fairly constant.†

*** The method of obtaining the alcoholic extractives preparatory to the action of sodium hypobromite was similar to that pursued in the estimation of urea in blood. (See foot-note, p. 119.)**

† In another case where a similar experiment was made, but in which the rate of infusion was much slower (2 cc. per minute), the urea rose to .648 per cent in the muscles, to .808 per cent in the liver and to .593 per cent in the brain.

TABLE XI.—Nitrogen, by Action of Sod. Hypobromite upon Alcoholic Extractives, of Muscle, Liver and Brain.

	Muscle.				Liver.				Brain.			
No.	Nature of experiment.	No. of hours.	Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.	Normal dog's muscle. Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.	Nature of experiment.	No. of hours.	Percentage of hydrogen by action of sod. hypobromite upon alcoholic extractives.	Normal dog's liver. Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.	Nature of experiment.	No. of hours.	Percentage of nitrogen by action of sod. hopobromite upon alcoholic extractives.	Normal dog's brain. Percentage of nitrogen by action of sod. hypobromite upon alcoholic extractives.
1	Double nephrectomy.	48	.098	.028	Double nephrectomy.	48	.048	.0075	Double nephrectomy.	48	.125	.048
2	"	48	.087	.029	"	48	.104	.0098	"	60	.132	.030
3	"	48	.125	.019	"	48	.091	.0102	"	60	.138	
4	"	60	.125	.036	"	60	.107	.....	R. ureter tied, L. renal tied.	36	.091	
5	"	60	.158	.035	"	82	.061	.....	Both renals tied.	36	.053	
6	"	82	.230	.033	Both ureterstied.	36	.146					
7	Both ureters tied.	36	.152	.034	Both renals tied.	36	.047					
8	R. ureter tied, L. renal tied.	36	.084									
9	Both renals tied.	36	.020									

Average: Nos. (1–6) muscle = .137 " Normal " = .031

Average: Nos. (1–5) liver = .082 " Normal " = .0092

Average: Nos. (1–3) brain = .132 " Normal " = .039

The results from the organs of nephrectomized dogs are mainly interesting when compared with the results similarly obtained from the organs of normal dogs. It is doubtful whether the nitrogen derived from the normal organs, especially in the case of the muscle, is due to the presence of urea. Thus Nencki and Kowarski* by employing phosphotungstic acid as a precipitant in the aqueous extract of large quantities of dog's muscle, obtaining an alcoholic extract, and eventually applying a delicate color test by the use of orthonitrobenzaldehyde and phenylhydrazin, arrived at the conclusion that urea is not a normal constituent of the muscle.

Few, if any, of the nitrogenous extractives except urea are wholly decomposed with liberation of nitrogen by the action of alkaline hypobromites, and upon most of the nitrogenous extractives the action of sodium hypobromite with liberation of nitrogen is slight. This favors the view that the increased nitrogen after nephrectomy is due to urea.

* *Arch. f. exp. Path. u. Pharm.*, 1895, xxxvi, 395.

ON THE INFLUENCE OF FASTING UPON THE BACTERICIDAL ACTION OF THE BLOOD.

By S. J. MELTZER, M. D., and CHARLES NORRIS, M. D.

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In the following we shall report the results of some experiments which we have made to ascertain the influence of fasting upon the bactericidal action of the blood. We were led to this line of experimentation by the casual observation made in our investigation of the bactericidal action of the lymph from the thoracic duct of the dog,* that our parallel experiments with the blood did not show such an intense germicidal effect as most other writers on this subject had observed. It occurred to us that the weaker bactericidal action observed by us may have been due to the fasting of our animals. In order to compare satisfactorily the number of colonies in the different plates, we had to obtain lymph free from the misleading fine droplets of chyle, and, therefore, our dogs were allowed to fast for 30 to 40 hours previously to their being operated upon. The investigations of other writers have not had this motive, and we may presume that in their experiments the blood was obtained within a few hours after feeding the animals.

The assumption that inanition might reduce the bactericidal action of the blood seemed to us plausible for the following reasons: It is now generally conceded that the bactericidal powers of the blood are generated in some way by the leucocytes. The hyperleucocytosis of digestion is a well established fact, and on the other hand, Luciani† found in a fasting human being (Succi) and Rieder‡ in fasting dogs a marked hypoleucocytosis. It is therefore reasonable to assume that the bactericidal elements are increased also during digestion, and

* Meltzer and Norris, *Journal of Experimental Medicine*, 1897, ii, 701.

† Luciani, Das Hungern, Hamburg and Leipzig, 1890.

‡ Rieder, Beiträge zur Kenntniss der Leukocytose u. s. w. Leipzig, 1892.

decreased in a state of inanition. Furthermore, if we consider the bactericidal elements as the natural protectors of the body against infection our hypothesis would coincide with the generally acknowledged fact, that a poorly nourished body is more apt to be attacked by an infectious disease than a well-fed one, a fact which finds support in the experiments of Canalis and Morpurgo,* showing that starving pigeons lose their natural immunity from the infection with anthrax bacilli.

To test the validity of this hypothesis was the object of our experiments. We restricted our experiments to dogs and the typhoid bacillus. The blood was obtained under the usual precautions either from the femoral or the carotid arteries under cocaine anæsthesia. The comparisons were made between specimens of blood from the same dog and similar arteries under different conditions of feeding. Serum and blood defibrinated by shaking with glass beads were examined in each experiment. The bactericidal power was studied by the Buchner method, *i. e.* a quantity of blood or serum was inoculated with the typhoid bacillus; from this plates were made immediately after inoculation, and then one hour, two hours, etc., after inoculation other plates were made, and the number of colonies on the different plates counted. In some of the experiments two series of observations were made; one with serum kept at 37° C., the other with serum kept at room temperature. We were often compelled by outside reasons to keep the blood two days or longer in the ice-box before the bactericidal power could be tested, but then with few exceptions the blood of the well-fed and of the fasting animal were kept under exactly the same conditions.

Exp. B[1]. Medium-sized male dog. Fed twice daily for two days. Nov. 4, a few hours after feeding, blood was taken from the right femoral artery. The defibrinated blood and the serum were kept in the ice-chest for 3 days, then inoculated and plated with the following result:

37° C.	Immediate.	1 hr.	6½ hrs.	25 hrs.	4 days.
Blood	5899	42	4	34916	....
Serum	1499	90	0	29	40000

* Canalis and Morpurgo, *Fortschr. d. Med.*, 1890, viii, 693 and 729.

Exp. B[2]. The same dog, after fasting for five days, on Nov. 9 (previous wound having healed per priam) was operated upon and blood was taken from the left femoral artery. Blood and serum kept in ice-box for 20 hours, then inoculated and plated.

37° C.	Immediate.	1 hr.	3½ hrs.	25 hrs.
Blood	3445	1197	60	6169
Serum	2210	496	60	15992

In this experiment there seems to be a slight difference in favor of the blood from the well-fed animal, although it was kept longer in the ice-box than the blood from the fasting animal.

Exp. C[1]. Small female dog fed a few hours before operation, Nov. 17; blood taken from the right femoral artery; blood and serum kept 40 hours in ice-chest, then inoculated and plated.

37° C.	Immediate.	1 hr.	3½ hrs.	6½ hrs.	24 hrs.
Blood	2316	169	9	4	27
Serum	689	79	19	15	194
21° C.					
Blood	1012	567	265	69	9
Serum	625	418	116	90	12

Exp. C[2]. Nov. 22 from the same dog, the previous wound having healed, fasting (without water) since the first operation, blood was taken from left femoral artery; the blood and serum were kept 48 hours in ice-box, then inoculated and plated.

37° C.	Immediate.	1 hr.	3½ hrs.	6 hrs.	24 hrs.
Blood	4086	583	43	8	81
Serum	5633	1155	123	4	390
21° C.					
Blood	3079	1706	678	413	185
Serum	5194	2234	1303	483	68

In this experiment there is apparently no difference between both kinds of blood and serum. Six hours after inoculation at 37° C., there is about the same reduction in all the specimens of the blood and the serum, though there was a higher initial number of colonies in the blood and the serum taken from the starved animal. The fasting apparently did not reduce the bactericidal power of the blood.

Exp. D[1]. Large dog fasted five days, no water; Dec. 1 blood was obtained from right femoral artery, blood and serum kept on ice for 4 days, then inoculated and plated.

37° C.	Immediate.	1 hr.	3 hrs.	6 hrs.	28 hrs.	3 days.
Blood	3831	572	84	14	∞	∞
Serum	2046	471	104	21	∞	∞
21° C.						
Blood	2814	1653	922	336	?	
Serum	1552	1701	662	381	651	

Exp. D[2]. The same dog well fed since last operation, Dec. 17, blood from left femoral artery, blood and serum 4 days in ice-chest, then inoculated and plated.

37° C.	Immediate.	1 hr.	3 hrs.	6 hrs.	28 hrs.
Blood	1807	286	81	23	∞
Serum	954	265	54	3	∞
21° C.					
Blood	1219	948	579	286	328
Serum	3768	588	445	312	1817

In this experiment the dog fasted first and was then fed well for over two weeks before blood was again taken; the result obtained was the same as in the foregoing experiments, and, to say briefly, as in all the previous experiments. Our hypothesis did not stand the test of the experiments; these have shown conclusively that five days' fasting did not affect the bactericidal power of the blood as tested with the typhoid bacillus, in the slightest degree. There seemed to be no difference in the bactericidal action of the blood, no matter whether it was taken from a well-fed or even over-fed dog or from an animal in a state of complete inanition.

We might perhaps explain this surprising fact in the following manner: According to Buchner the degree of the bactericidal action of the body-fluids depends upon two opposing factors; the alexines, which are generated by the leucocytes, and the nutritive character of the fluid. There can be no question that fasting impoverishes the blood. If we now assume that feeding or fasting increases or diminishes the nutritive capacity of the blood to the same degree as hyper- or hypoleucocytosis is obtained, it would then be quite natural that the bactericidal action should always remain the same, for when numerator and denominator are multiplied or divided by the same number, the value remains unchanged.

As to any apparent incompatibility of our results with the common assumption that inanition favors infection, we must in the first place

bear in mind that our present results apply only to the bactericidal action of the blood of the dog upon the typhoid bacillus; with other animals and with other microörganisms the conditions may be different. Canalis and Morpurgo could not by starvation influence the immunity of the rat from anthrax, although the immunity of the pigeon was abolished. On the other hand the bactericidal properties of the blood are not the only defensive elements of the body against infection. Other factors, notably the body cells, are concerned, and it may well be that the powers of resistance of the latter may suffer to a considerable measure through the starvation of the animal.

GLYCOSURIA IN DIPHTHERIA.

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AND
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The occurrence of a transitory glycosuria in diphtheria has been noted by only a few observers. Sanné† in his monograph on diphtheria says: "The analyses made by Fouris to determine the presence of sugar (in the urine) have given only negative results." Dickinson‡ reports: "I have examined many cases of croup in which the breathing has reached the extreme of difficulty without finding a trace of sugar in the urine, with one exception—a girl seven years old." Simon§ in his Clinical Diagnosis speaks of transitory glycosuria in diphtheria. In a personal letter Dr. Simon says: "A few years ago while examining a series of urines with the phenylhydrazin test I accidentally came across a urine from a case of diphtheria in which the osazon needles were present in fairly large numbers. Two years ago I had occasion to examine the urine of thirty-two cases of diphtheria, all of which were of moderate severity. A positive reaction was obtained with the phenylhydrazin in four. Three of these four were treated with antitoxin. The amount of sugar in each case was small and persisted, if I remember, only one to two days." A Rumbold‖ remarks that in diphtheria during convalescence glycosuria has been demonstrated on rare occasions, but gives

* Dr. Hibbard died as the result of an elevator accident in St. Louis in July, 1898.

He was graduated from the Harvard Medical School in 1895 and subsequently served for two years as Assistant Resident Physician in the South Department of the Boston City Hospital. He published several valuable contributions to our knowledge of the infectious diseases of children. He was an enthusiastic and untiring student, who gave promise of unusual success as a clinical investigator.—W. T. C.

† Gillis' English translation, 1886, p. 185.

‡ W. H. Dickinson. Diseases of the kidney and urinary derangements, Pt. I; Diabetes, p. 20, London, 1875.

§ Charles E. Simon. A manual of clinical diagnosis, p. 369. Philadelphia and New York, 1896.

‖ *Wien. klin. Wochenschr.*, 1894, p. 83.

no reference and no observations. Coleman* speaks of a transitory glycosuria in diphtheria.

Paul Binet† found in diphtheria a positive reaction with the phenylhydrazin test in 29 out of 70 cases. Dividing these into mild and severe forms, there were 32 of the former with only 2 positive reactions, while of the 38 severe cases there were 27 with positive result. Of 19 cases of laryngeal diphtheria 11 showed the characteristic osazon crystals. With Fehling's test 37 of the 70 cases gave a green or a greenish-yellow reaction. The glycosuria was frequently associated with albuminuria.

The following observations are the result of about one thousand analyses of the urine for sugar by Fehling's test from diphtheria patients of the South Department of the Boston City Hospital from October 1, 1897, to January 1, 1898. Specimens of urine were examined from all old patients in the wards and from new ones as they were admitted. The analyses were made every three to five days. A daily test was usually made in those cases that showed a positive reaction or were very sick.

Tests.—*Fehling's:* 7 cc. of the mixed solution were boiled in a test tube and to this 4 cc. of cold urine were added. If the urine contained albumin, this was first separated by heat and filtration. The urine and Fehling's solution were well shaken and allowed to stand until the next day without further heating. The presence of a yellowish precipitate in the bottom of the tube was considered as a positive reaction. In any instance where there was any question as to the result of the reaction it was called negative.

Most of the positive results with Fehling's method were confirmed by the *phenylhydrazin test*, which was performed in these observations in the following manner:‡ To 7 cc. of filtered urine, free from albumin, 3 or 4 drops of phenylhydrazin and the same quantity of 50 per cent acetic acid were added. The mixture, after being well shaken in a test tube and kept in a water bath for at least one hour, was allowed to settle until the next day. The brownish sediment was examined under

* Article on Diabetes in Loomis and Thompson's System of Practical Medicine by American Authors. Vol. iii, p. 829, New York and Philadelphia, 1898.

† *Rev. méd. de la Suisse Rom.*, 1892, xii, 69.

‡ Foster's Physiology, part v, p. 104.

the microscope and the presence of the characteristic phenylglucosazon crystals made a positive reaction.*

In the following analyses only those cases that gave a positive result both by Fehling's and by the phenylhydrazin tests were considered as instances of glycosuria. Doubtless many of the urines, not in the table, which reduced the copper solution contained glucose, but for various reasons the phenylhydrazin test was not applied to some of them.

Table I gives the results of Fehling's tests in 230 cases of diphtheria, in all of which the Klebs-Loeffler bacillus was found. Some of these patients were well along in convalescence; other urines were examined only once or twice. The cases are classified according to the presence or absence of pseudo-membrane and its location.

TABLE I.—Results of Fehling's Test.

	RECOVERED.			DIED.			TOTAL.		
	No. of cases.	No. of positive reactions.	Percentage of positive reactions.	No. of cases.	No. of positive reactions.	Percentage of positive reactions.	No. of cases.	No. of positive reactions.	Percentage of positive reactions.
No membrane	22	0	0	1	0	0	23	0	0
Membrane on tonsils	98	8	8	3	1	33	101	9	9
In nose	5	2	40	0	0	..	5	2	40
In larynx	11	2	19	2	2	100	13	4	31
Tonsils and nose	45	12	27	6	5	83	51	17	33
Tonsils and larynx	19	10	53	5	4	80	24	14	58
Nose and larynx	5	3	60	1	1	100	6	4	67
Tonsils, nose and larynx	3	2	66	4	4	100	7	6	86
Total	208	39	19	22	17	77	230	56	25

It is seen that in the cases without false membrane there was no positive reaction, and that, in general, the more extensive the membrane, the more frequently a positive reaction was found. A reaction was noted in 19 per cent of the recoveries, in 77 per cent of the fatal cases and in 25 per cent of all the cases.

In a second series of 96 cases the positive reactions by Fehling's

* We wish to thank Dr. J. Bergen Ogden, Assistant in Clinical Pathology in the hospital, for his help and advice in these observations.

method were confirmed by the phenylhydrazin test. These analyses were begun during the first week of the patient's stay in the hospital, and were followed for at least a fortnight by as many as three examinations. In other words, those cases of Table I are omitted in which a reaction by Fehling's test was not confirmed by phenylhydrazin, and also those not examined within the first week of the illness.

TABLE II.—Results of Fehling's and the Phenylhydrazin tests.

	RECOVERED.			DIED.			TOTAL.		
	No. of cases.	Cases with sugar.	Percentage of sugar cases.	No. of cases.	Cases with sugar.	Percentage of sugar cases.	No. of cases.	Cases with sugar.	Percentage of sugar cases.
No membrane.....	10	0	0	0	0	.....	10	0	0
Membrane on tonsils..	37	6	16	2	1	50	39	7	18
In nose.....	2	1	50	0	0	.....	2	1	50
In larynx	4	2	50	1	1	100	5	3	60
Tonsils and nose.....	21	8	38	2	2	100	23	10	44
Tonsils and larynx....	9	5	55	1	1	100	10	6	60
Nose and larnyx.....	3	3	100	1	1	100	4	4	100
Tonsils, nose and larynx	1	0	0	2	2	100	3	2	66
Total.....	87	25	28	9	8	88	96	33	34

Table II shows, in an even more marked degree, the same results as those in Table I, and also that a transitory glycosuria, of at least as much as 0.5 gramme of glucose to the litre of urine,* is not at all uncommon in the course of diphtheria. There were 15 cases which gave a positive Fehling's reaction that are omitted from Table II, as a phenylhydrazin test for some reason was not made; so that the percentage given is doubtless too low. The greater the surface involved by the diphtheritic process the greater the frequency of sugar. Again, a glycosuria is three times as common in the fatal cases as in those that recover.

The time at which Fehling's test was first positive varied from the second to the eighteenth day. In 35 cases a positive reaction was found on the first analysis, and in 21 on the second, or on some subse-

* F. D. Beane. The comparative clinical value of several tests for glucose in the urine. *New York Med. Jour.*, 1893, lvii, 12.

quent examination. In two cases five negative results were obtained before a positive one. In the 44 positive cases that were tested by Fehling's solution within the first week of the illness, the reaction was found within that time in all but 8, and in the latter it was obtained during the second week. In one instance after a reaction had appeared on the eighth day it was followed by six negative results, and then on the twenty-first day by another positive reaction, confirmed by the phenylhydrazin test. In another case after a glycosuria had been shown with Fehling's solution, and again on the seventh and fourteenth days three negative results followed; a positive result appeared on the twenty-second and twenty-sixth days, and a negative on the twenty-eighth day.

The duration of the glycosuria varied from one day to several weeks. Seven patients left the hospital with sugar still present in the urine. In a case of unresolved pneumonia, following a triple attack of laryngeal diphtheria that had been in the hospital for five months, glycosuria was found on ten successive analyses during the sixth month of the illness.

An immediate reaction with Fehling's test occurred 16 times in the 56 positive cases. In the others the reaction was seen at the end of 24 hours. In three cases the immediate reaction was observed from one to three days after one that appeared only at the end of a day. There were three quantitative estimations with Fehling's solution, with the following results: 1.5, 2, and 3.3 per cent, the first two of these being in fatal cases. Of the 16 patients with an immediate reaction eight or 50 per cent died. It would thus seem that an immediate reaction with Fehling's test is of serious prognostic import, more so than the presence of albumin in the urine of a diphtheria patient.

The relation of glycosuria to albuminuria is shown in Table III, which gives the number of cases in which the reaction for sugar was absent, the number in which it was obtained and the percentages.

TABLE III.—Relation of Glycosuria to Albuminuria.

Albumin.	No sugar.	Sugar.	Percentage.
None	58	0	0
Slight trace	139	16	10
Distinct trace	25	11	31
0.125 per cent or more	2	8	80

These results confirm the observation of Binet that the glycosuria of diphtheria is usually associated with albuminuria, without there being necessarily any definite relation between them, and also that sugar is oftener present in the severer cases of albuminuria.

Some additional observations on scarlet fever both with and without diphtheria were made by Mr. C. H. Dean, the clinical clerk in the scarlet fever wards, who made 920 Fehling's tests in 129 cases of scarlet fever. Of 93 patients, in whom no diphtheria bacilli were found, 16 gave positive reaction once. One of these patients was pregnant, and therefore this case should be eliminated, as the reduction was probably due to lactose, the phenylhydrazin test giving phenyllactosazon crystals. This makes the positive reactions about 16 per cent. 4 patients had such suspicious looking membranes that they received antitoxin, and the urine of 3 of these gave a yellow reaction. Of 36 cases of scarlet fever with positive cultures of diphtheria bacilli from the nose or throat, 11 or about 30 per cent gave a positive reaction. In the last series of 37 cases, 17 received antitoxin and 6 of these showed a reduction of the copper, which gives about the same percentage of reactions as in the cases which did not receive antitoxin. All the positive results in scarlet fever occurred only once, save in 3 cases which also had diphtheria. In one the reaction appeared on two occasions with an interval between them of 22 days. In the other case it was found on three successive examinations during a period of 10 days. In the third, a fatal case, it was present in the last two days of life. It is to be regretted that these reactions were not confirmed by the phenylhydrazin test.

In the scarlet fever patients who also had diphtheria bacilli, the percentage of positive reactions was about twice that found in scarlet fever alone. This observation would seem to indicate that the existence of diptheria is a causal factor in the production of a reducing agent in the urine.

Eliminating the case of lactosuria, there were 21 scarlet fever patients who received antitoxin, and 107 who did not, with 9 positive reactions or 43 per cent in the former, and 17 reactions or 16 per cent in the latter. In other words, a reaction was over two and a

half times more frequent in those patients who received antitoxin than in those who did not. From this it would appear that the administration of antitoxin contributed to the formation of a substance in the urine which reduces the copper in Fehling's solution.

9 cases of measles were examined several times by Fehling's test, each with negative results, save in one instance, a patient who was nursing a child. The reduction here was doubtless due to lactose, as no reaction was obtained with the phenylhydrazin test.

In sixty-eight cases of tonsillitis the urine was examined on one or more occasions. A positive result was obtained only once, but unfortunately in this instance, no confirmatory phenylhydrazin test was made.

As almost every diphtheria patient received more or less antitoxin the question arose, Was this method of treatment responsible for the transitory glycosuria found in our patients? In order to clear up this point the urine of 20 patients who had false membrane and the Klebs-Loeffler bacillus was examined prior to the serum injection with the following results:

Fehling's test.—15 cases negative; 1 urine gave a slight reduction in 24 hours; 3 cases gave a good reduction in 24 hours.

Phenylhydrazin test.—10 cases had no crystals; 6 cases had a few osazon crystals; 4 cases had many crystals.

The percentage of positive Fehling's reactions in these few cases is about the same as in Series I, while the positive results with the more delicate tests, as was to be expected, are more frequent than in Series II. These results warrant the conclusion that the antitoxin treatment cannot be considered as the full explanation of this symptom in diphtheria.

To decide whether diphtheria antitoxin injected into patients may produce a glycosuria 10 individuals were given antitoxin in whom the two tests for glucose had proved negative before the injection, and the urine was examined afterward. The results of these observations are given below in detail:

No. I. Male 19 years old. Admitted Dec. 30 with tonsillitis. At 4 and 10 P. M. Fehling's and phenylhydrazin tests were negative. At

11 P. M. given 20 cc. antitoxin. Examinations at 4 and 9 A. M. the following morning were negative by Fehling's, but there were a few doubtful sheaf-like crystals by the latter test. Dec. 31, at 9.30 A. M., given 20 cc. antitoxin. At 4 P. M. and 1 A. M., Jan. 1, Fehling's test was negative. At 9 A. M. a very few doubtful osazon crystals. Same found on Jan. 2. The results of this experiment are called negative as the crystals were so few and of a questionable nature.

No. II. Male 22 years old. Admitted Nov. 5 with a severe diphtheria of throat; given first day 20 cc. antitoxin; the urine next morning was positive to Fehling's test. From the second to the fourth day received 55 cc. more antitoxin. On the fifth day both Fehling's and the phenylhydrazin tests were positive. On the eighth day both were negative. During the following month had eight negative results by Fehling's test. On Dec. 16 was given 20 cc. antitoxin; the urine was examined every 12 hours for the next 5 days, then once a day for 5 days by both tests, with negative result.

No. III. Boy, 13 years, admitted Nov. 18 with tonsillar diphtheria. He received 60 cc. antitoxin during the first two days. Six examinations with Fehling's solution were made in the first 4 weeks with negative result. Dec. 20 phenylhydrazin test negative. Given 20 cc. antitoxin on this date. The examination by Fehling and the phenylhydrazin tests were negative on the two days following.

No. IV. Girl, 10 years, admitted Nov. 15 with diphtheritic tonsillitis, so mild that antitoxin was not given. Four examinations in 3 weeks, by Fehling's test, were negative. On Dec. 28 given 20 cc. antitoxin. The phenylhydrazin test just before antitoxin was given was negative, but six hours afterwards positive; there were numerous typical crystals. The next three days many crystals of osazon appeared, and after that for a week only a very few or none were seen. Fehling's tests were negative, with possibly one doubtful reaction on Jan. 5.

No. V. Girl, 7 years, admitted Oct. 30 with a mild attack of diphtheria; was given no antitoxin. On Nov. 3 Fehling's and the phenylhydrazin tests were both positive. From Nov. 6 to Dec. 28 there were 8 negative copper tests and on the last date there were no osazon crystals. On Dec. 28 were given 20 cc. antitoxin. The following morning there was a slight greenish-yellow discoloration of the Fehling's solution and numerous phenylglucosazon crystals were seen. The following 12 examinations by Fehling's test were all negative save one doubtful one. The phenylhydrazin test was positive for the next 6 analyses, then negative.

No. VI. Girl, 7 years. Admitted Dec. 7 with slight nasal diphtheria; received no antitoxin. On the next day had an immediate reaction to Fehling's test, followed by five negative results during the next week. Dec. 18 given 10 cc. antitoxin. At that time and the next day the urine was negative to both the Fehling's and phenylhydrazin tests, but on the following day it was positive in the morning and evening to the phenylhydrazin test and afterwards negative. Fehling's tests were all negative.

No. VII. Girl, 7 years, admitted with a mild attack of scarlet fever on Dec. 27. Urine examined daily from Jan. 13 to 19. Negative by both tests. On the last date given 20 cc. antitoxin. Analyses twice a day for two days, then daily for five days with negative results.

No. VIII. Boy, 6 years, taken ill with scarlet fever Dec. 15. Similar tests were made, on the same date, as in No. VII. The morning after the antitoxin injection Fehling's test was positive and there were many phenylglucosazon crystals seen. There was a slight reaction that evening, afterwards negative.

No. IX. Girl, 9 years old, admitted with scarlet fever, Dec. 29. Experiment same as in No. VII. Results negative.

No. X. Boy, 7 years old, admitted Dec. 18 with scarlet fever. In this case, as in No. VII, Fehling's tests were positive, but with phenylhydrazin the results were negative.

A summary of the results of these experiments by the two tests are:

Case No.	Fehling's.	Phenylhydrazin.
I.	Negative.	Negative.
II.	Negative.	Negative.
III.	Negative.	Negative.
IV.	Negative.	Positive.
V.	Doubtful.	Positive.
VI.	Negative.	Positive.
VII.	Negative.	Negative.
VIII.	Positive.	Positive.
IX.	Negative.	Negative.
X.	Positive.	Negative.

Fehling's test was twice positive and the phenylhydrazin four times. Thus it would appear that antitoxin injections produce at times a glycosuria, but that usually it is so slight that it is shown only by the most delicate tests; on the other hand sugar is occasionally present in a sufficient quantity (0.05 per cent at least) to reduce Fehling's solution. This agrees with what was noted in regard to the

scarlet fever patients who received antitoxin (pp. 142-3). It should be added that this glycosuria lasts only for a few days and should not be considered as an argument against the use of antitoxin. The significance of a transitory glycosuria after antitoxin would appear to be about the same as that belonging to the alimentary glycosuria which may occur after the ingestion of certain foods or drugs, or after drinking beer, as Krehl* has recently shown.

Binet† gives as the cause of the glycosuria in diphtheria an "asphyxie toxique," due for the most part to a simple mechanical obstruction of the respiratory passages. Von Bager‡ reports an instance of glycosuria in a patient troubled with a nasal obstruction which disappeared after an operation. Tyson§ says: "Embarrassed respiration, whether due to strangulation or inhalation of irrespirable gases is capable of producing glycosuria in dogs and rabbits, although the symptom has rarely been shown in those conditions in human subjects." Of 19 cases of intubation, that is, of patients with severe laryngeal obstruction, fourteen, or 73 per cent, had glycosuria. This result would make somewhat plausible the supposition that diminished oxidation in consequence of the greater or less interference with respiration has a causal connection with the glycosuria of diphtheria. It seems to us, however, reasonable to attribute this symptom in diphtheria to the action of the toxins of the Klebs-Loeffler bacillus. Interference with respiration may be an occasional cause, but it will not account for those cases in which there is no trouble with the breathing. Inasmuch as irritation of the vagus nerve and various lesions of the nervous system may be followed by glycosuria, and as degenerative changes in the peripheral and central nervous system are found in most cases of diphtheria, it is possible that these changes in the nervous system in diphtheria may cause glycosuria. Or it may be that the diphtheria toxins may act as phloridzin|| does in producing glucose in the urine.

* *Centralbl. f. inn. Med.*, 1897, No. 40.

† Loc. cit., p. 87.

‡ *Centralbl. f. d. Krankheiten d. Harn- u. Sexual-Organe*, vi, 61.

§ A treatise on Bright's disease and diabetes, p. 239, Philadelphia, 1881.

|| Lectures on certain aspects of diabetes, by T. B. Futcher in the *New York Med. Jour.*, Nov. 6, 1897.

CONCLUSIONS.

(1) There is a transitory glycosuria in diphtheria, which is found frequently in the severe cases and is usually present in the fatal ones.

(2) This glycosuria is often associated with albuminuria.

(3) Injections of diphtheria antitoxin are occasionally followed for a few days by a slight glycosuria.